

PATIENT INFORMATION		REFERRAL INFORMATION	
NAME JANE DOE		CLINIC NAME CLINIC X	
ETHNICITY ETHNICITY X		CLINIC ID XXXX	
DATE OF BIRTH XX/XX/XXXX		REFERRING CLINICIAN Dr. XXXX	
TEST INDICATIONS XXXXXXXX		CLINIC EMAIL XXXXXX@email.com	

SAMPLE INFORMATION			
ORDER NUMBER XXXX	LAB NUMBER XXXX	DATE OF COLLECTION XX/XX/XXXX	DATE RECEIVED XX/XX/XXXX

RODINIA INFERTILITY TEST

PANEL SELECTED* FEMALE INFERTILITY PANEL MALE INFERTILITY PANEL

ADD-ON PANEL SELECTED YES NO

TEST RESULTS

No Clinically Significant Findings / Variant Detected

No numerical and structural abnormalities were detected. No pathogenic or likely pathogenic variants were detected in the genes tested. This interpretation is based on the clinical information provided and the current understanding of the molecular genetics of these conditions. The result should be evaluated in the context of all clinical findings and patient history. The results of the haemophilia and NAIT panel should be further evaluated by the referring clinician. Genetic counselling is recommended for all individuals undergoing genetic testing.

ADD-ON PANEL: HAEMOPHILIA and NAIT RESULTS

GENE	ALTERNATIVE NOMENCLATURE	VARIANT	RESULT	COMMENT
F5 (Factor V Leiden)	R506Q	NM_000130.4(F5):c.1601G>A (p.Arg534Gln)	Homozygous, Normal Genotype	Factor V Leiden variant is associated with thrombophilia due to activated protein C resistance. Studies suggest that the relative risk for venous thrombosis associated with the factor V Leiden variant, in the absence of other acquired or environmental predispositions, is approximately 4- to 7-fold for heterozygotes and 50-fold for homozygotes.
F5 (Factor V R2)	H1299R	NM_000130.4(F5):c.3980A>G (p.His1327Arg)	Heterozygous	This polymorphism in factor V gene has been reported to be a possible risk factor for the development of venous thromboembolism (VTE). It was found in association with factor V (F5) gene mutations in family members with venous thrombosis.

				<p>homozygosity for factor V1050Q and PAI2 conferred a 2- to 4-fold increase in the relative risk of venous thromboembolism compared with factor V1050Q alone.¹</p>
F13A1 (Factor XIII)	V34L	NM_000129.3(F13A1):c.103G>T (p.Val35Leu)	Homozygous, Normal Genotype	<p>This variant has been reported to confer protection against venous thromboembolism and there is evidence that the homozygous state for the V34L mutation is a strong protective factor against venous thromboembolism.¹</p>
ITGB3 (HPA-1)	L33P	NM_000212.2(ITGB3):c.176T>C (p.Leu59Pro)	a/a	<p>Hemolytic uremic syndrome (HUS) is a thrombotic microangiopathy with systemic complications including a proliferative glomerulonephritis and a microangiopathic process characterized by thrombocytopenia, microangiopathic hemolytic anemia, and acute renal failure. The gene encoding von Willebrand factor is located on chromosome 12 and the mutation allele is known as "V1". The alternative allele of von Willebrand factor is known as "V2". The von Willebrand factor protein product may not react with platelets, resulting either in more severe risk assessment and better prophylactic management. Hemolytic uremic syndrome (HUS) is a thrombotic microangiopathy that occurs in one in 1,000-1,500 live births and is the most common cause of severe thrombocytopenia and intravascular hemolysis in birth infants. The disease spectrum may vary from mild to severe thrombocytopenia, intravascular hemolysis (ICH). A diagnosis of hemolytic uremic syndrome (HUS) should be considered for any neonate with unexplained thrombocytopenia. Once the diagnosis is made, it is known that all subsequent pregnancies are at risk for severe disease.¹</p>
GP1BA (HPA-2)	-	NM_000173.7(GP1BA):c.482C>T (p.Thr161Met)	a/b	
ITGA2B (HPA-3)	I843S	NM_000419.5(ITGA2B):c.2621T>G (p.Ile874Ser)	a/a	
ITGB3 (HPA-4)	R143Q	NM_000212.2(ITGB3):c.506G>A (p.Arg169Gln)	a/a	
ITGA2 (HPA-5)	-	NM_002203.4(ITGA2):c.1600G>A (p.Glu534Lys)	a/a	
ITGB3 (HPA-6)	R489Q	NM_000212.2(ITGB3):c.1544G>A (p.Arg515Gln)	a/a	
SERPINE1 (PAI-1 4G/5G)	4G/5G	NM_000602.5(SERPINE1):c.-820G[(4_5)]	4G/5G	<p>Studies indicate that 4G allele of the polymorphism was found to be associated with higher plasma PAI-1 activity.¹ The 4G/4G genotype was associated with a greater risk of thrombosis both in cryptohemolytic thrombophilia patients and in idiopathic DVT (deep vein thrombosis) patients. The greater frequency of 4G allele in cryptohemolytic thrombophilia patients with respect to controls was statistically significant. PAI-1 4G/5G polymorphism may influence PAI-1 expression and thrombotic risk in patients with acquired thrombophilia.¹</p>
MTHFR	C677T	NM_005957.5(MTHFR):c.665C>T (p.Ala222Val)	Heterozygous	<p>There are two common polymorphisms in the MTHFR gene,</p>

MTHFR	A1298C	NM_005957.4(MTHFR):c.1286A>C (p.Glu429Ala)	Homozygous, Normal Genotype	MTHFR C677T (rs1801313) and MTHFR A1298C (rs1801311) MTHFR C677T polymorphism is thought to increase the risk of venous thromboembolism (VTE). Homozygosity of C677T is associated with elevated plasma homocysteine levels and folate deficiency. The MTHFR C677T variant results in a thermolabile protein with enzymatic activity that is decreased by 35% in the heterozygous state (CT genotype) and by 70% in the homozygous state (TT genotype). Methylene tetrahydrofolate reductase (MTHFR) is a key enzyme in the methylation pathway. In the heterozygous state (CT genotype), especially in pregnant women, we observe an increased risk of VTE. Homozygous individuals does not have this risk. MTHFR polymorphisms are associated with MTHFR genotyping showing more consistent association with VTE risk. A meta-analysis of several studies has demonstrated that both maternal and paternal MTHFR gene C677T and A1298C variants are associated with recurrent pregnancy loss ¹ .
ACE (ACE (I/D))	ACE/ID polymorphism	NM_000789.3(ACE):c.2306-117_2306-116insAF118569.1:g.14094_14382	D/D	A codon insertion/deletion polymorphism within the angiotensin-converting enzyme gene (ACE I/D) has been reliably associated with substantial differences in the plasma and tissue angiotensin-converting enzyme (ACE) activity in a consistent (additive) fashion not only in persons of European descent but also in other populations. Individuals carrying the D allele have higher ACE activity, which has been proposed as an intermediate phenotype of potential relevance for the development of high blood pressure and tubulovascular sclerosis ¹ .
APOB	R3500Q	NM_000384.3(APOB):c.10580G>A (p.Arg3527Gln)	Homozygous, Normal Genotype	The R3500Q mutation in the apolipoprotein B gene, which is responsible for familial defective apolipoprotein B-100, causes severe hypercholesterolemia and increases the risk of premature heart disease ¹ .
APOE	R158C	NM_000041.2(APOE):c.526C>T (p.Arg176Cys)	E3/E3	The ApoE gene is a polymorphic glycoprotein consisting of 3 codon alleles, ε2, ε3, and ε4, and is able to generate 6 different genotypes (ε2/ε2, ε2/ε3, ε2/ε4, ε3/ε3, ε3/ε4, and ε4/ε4). Many studies assessing the effect of the ApoE genotype on plasma lipids have indicated that the presence of
APOE	C112R	NM_000041.4(APOE):c.388T>C (p.Cys130Arg)		

				<p>The c2 allele is associated with elevated total cholesterol levels but that the presence of the c2 allele is associated with decreased levels of cholesterol. Thus, the ApolE genotype affects the progression of atherosclerosis, which is the main pathway underlying the ischemia-related cerebrovascular disease. Although the exact mechanism responsible for the association between ApolE polymorphism and CVD risk remains unclear, it is speculated that the c2 allele enhances lipid deposition in blood vessels. Thus, one might expect individuals to exhibit an increased susceptibility to CVD. Furthermore, the ApolE c2 allele was associated with an increased risk of developing hypertension, which may be a consequence for the association of this allele with an increased CVD.</p>
MTR		NM_000254.2(MTR):c.2756A>G (p.Asp919Gly)	Heterozygous	
MTRR	p.I49M:ATA>ATG	NM_002454.3(MTRR):c.66A>G (p.Ile22Met)	Homozygous for mutation	<p>homocysteine has received considerable attention as elevating in plasma and homocysteine has been implicated as a risk factor for vascular disease. There are two pathways in homocysteine metabolism, remethylation and transsulfuration, and three genes are involved, MTHFR, MTRR and MTR, in the homocysteine metabolism. The substitution effects of MTHFR 677T, MTHFR 1294A, MTR 2756A>G and MTRR 66A>G showed a higher risk of hyperhomocysteinemia, and this was aggravated by folic acid deficiency¹⁶.</p>
AGT	M235T	NM_000029.4(AGT):c.803T>C (p.Met268Thr)	Heterozygous	<p>There is strong evidence that deleterious variants of angiotensinogen-converting enzyme predispose to essential hypertension in humans. The substitution of threonine for methionine at amino acid position 235, showed the strongest linkage to the hypertensive phenotype and elevated plasma levels of angiotensinogen.</p>
AGTR1	A1166C	NM_031850.3(AGTR1):c.*86A>C	Homozygous for mutation	<p>An alanine,threonine (A/T) base substitution at position 1166 in the angiotensin II type 1 receptor gene is associated with the incidence of essential hypertension and increased coronary artery revascularization.</p>
GSTP1	GSTP1*B	NM_000852.4(GSTP1):c.313A>G (p.Ile105Val)	Homozygous, Normal Genotype	<p>The role of polymorphism in glutathione S-transferase (GST), involved both in antioxidant defense and in regulation of apoptosis signaling pathways in HF (heart failure), has been proposed. GSTP1-Ile105Val (rs11385) allele carriers</p>

				<p>rate of 1.7-fold increased HF risk than GUTP1-495/49 carriers. GUTP1 polymorphic variants may determine individual susceptibility to oxidative stress, inflammation, and endothelial dysfunction in HF¹¹.</p>
F2 Prothrombin	G20210A	NM_000506.5(F2):c.*97G>A	Homozygous, Normal Genotype	<p>F2 c.*97G>A (also known as c.20210G>A or G20210A) has been associated with increased risk for venous thromboembolism (VTE) variant has been observed in diverse ethnic backgrounds. As reported in meta-analyses have reported odds ratio of 1.1-1.2 for developing VTE versus thrombotic risk polymorphic inheritance of prothrombin G20210, heterozygous carriers (c.20210G, F2 c.143G>A and c.20210G) have been shown to increase pregnancy loss.</p>

METHODOLOGY/LIMITATIONS

Rodinia is a Laboratory Developed Test (LDT) from NIPD Genetics, a Medcover Company LMT for infertility testing. Genetic identification using DNA is performed using a standardized protocol and reported to technician & registration prior to DNA library preparation. DNA enrichment for the genes of interest is carried out using a solution-based hybridization method followed by next-generation sequencing (NGS). Sequence data is aligned to a reference genome and variants are identified using proprietary bioinformatics pipeline. Rodinia can be used for the identification of single nucleotide variants, small insertions and deletions (indels) and copy number variations (CNV). Variants are classified according to the criteria set by the American College of Medical Genetics and Genomics¹². Classification and interpretation of variants is performed using the VarSome Clinical website and is according to published knowledge at the time of testing. Variants which are classified as pathogenic, likely pathogenic are reported. Variants which have been detected and are classified as variants of uncertain significance (VUS), benign or likely benign are not reported. VUS will only be reported in cases of potential pathogenicity. The clinical significance of variants will not be reported. Genetic counseling for the clinical interpretation and significance of findings is recommended. A 'No clinically significant findings' indicates the absence of an inherited, de novo or somatic variant but does not guarantee that the individual does not have a genetic cause for his/her condition. A 'Clinically significant finding' indicates that a genetic change has been identified and that the individual will likely develop the condition. Results that are associated with trisomy 21 and XPT are listed in a separate table when applicable.

The test aims to detect variants relevant to the genes listed in Table 1 by targeting all coding exons and 20 bp of adjacent intronic sequence. Variants that fall outside of the targeted regions are not intended to be detected by this assay, unless otherwise noted. Exon-intron junctions (INDEL) and INDELs in the intron and other non-coding regions are not covered by this assay. Certain types of variants (CNVs and INDELs) in non-coding regions of selected genes that are of clinical significance are also included in the results. In cases where two variants are identified in a gene, the test does not distinguish whether these are on the same chromosome (in cis) or on different chromosomes (in trans). Certain types of genetic abnormalities such as inversions, translocations, polyploidy and epigenetic effects are not covered by this test. Certain sequence changes (CNVs and INDELs) in targeted regions containing repeats, sequences of high homology such as segmental duplications and pseudogenes, as well as regions of extreme GC-content may not be detected. The test is designed to detect CNVs at the gene level for all genes described below, unless otherwise noted, with high sensitivity and specificity. The test also detects CNVs up to a few exon level with lower sensitivity of the genes listed. All positive CNVs are confirmed with an orthogonal method. The test cannot detect CNVs up to a single or a few exon resolution at genomic regions with either low mappability or containing repeats, pseudogenes and extreme GC-content. The lack of disease-causing variants in the targeted genes does not exclude the possibility of a disease-associated syndrome. De novo chromosomal and/or structural variants (aneuploidies, copy number changes (CNVs), and structural variants (SVs)) can be detected by Rodinia test. Although the test is highly accurate there is still a possibility for false positive and false negative results.

Chromosomal Y microdeletions and Fragile X syndrome are performed using methodologies described below:
 Chromosomal Y microdeletions: Analysis of the azoospermia factor 1 (AZF) regions on the Y chromosome, which is associated with spermatogenic failure in the infertile men, is performed by multiplex ligation-dependent amplification (MLPA). This MLPA analysis detects deletions/duplications in AZFa, AZFb and AZFc regions. MLPA cannot detect any changes that lie outside the target sequence of the probes and will not detect copy number neutral inversions or translocations. Even when

MLPA did not detect any alterations, the possibility remains that although a change in that gene or its chromosomal region do exist but remain undetected - sequence changes (e.g. SNPs, point mutations, small indels) in the target sequence detected by a probe can cause false positive result. False positive and false negative results can also occur due to rare technical reasons.

Fragile X syndrome: Isolated DNA is amplified by the polymerase chain reaction (PCR) to determine the size of the CGG repeat within the FMR1 gene. PCR products are generated using a fluorescently labeled primer and sized by capillary gel electrophoresis. The interpretation is based on the following range of repeat sequences: Negative: <44 repeats, Intermediate: 45-64 repeats, Pre-mutation: 65-200 repeats, Full/mutated >200 repeats. Southern blot is not performed. Therefore, repeat expansion may not be able to detect size number beyond 200. The methylation status is not analyzed. False positive or false negative results may occur due to rare reasons that include rare genetic variants, mosaicism, blood contamination, bone marrow transplantation or other rare molecular events.

The Rodinia infertility test development and performance evaluation was carried out by NPD Genetic Public Company Limited, which is regulated under the Clinical Laboratory Improvement Act of 1988 (CLIA) as qualified to perform high-complexity testing. Rodinia is intended for clinical purposes and should not be regarded as investigational or for research. The test has not been cleared or approved by the U.S. Food and Drug Administration (FDA), which does not require this test to go through pre-market FDA review.

ADDITIONAL TECHNICAL SPECIFICATIONS

NI, MLPA: Deletion/Amplification analysis is not performed for these genes

ADDITIONAL INFORMATION / DISCLOSURE

Validation studies are carried out by NPD Genetic Public Company Ltd. The test will not identify all variants associated with the disorders tested. Although this test is highly accurate, there is still a small possibility for false positive or false negative results. This may be caused by technical and/or biological limitations, including but not limited to: mislabeled samples, inaccurate reporting of clinical/medical information, human technical errors, or other rare events. Some undetected genetic changes could be disease-related and are not covered by this Genetic testing is an important part of the diagnostic process. However, genetic tests do not always give a definitive answer. In some cases, testing may not identify a genetic variant even though one exists. This may be due to limited or no current medical knowledge or testing technology. Clinical correlation with other clinical data and tests is recommended. Results should always be considered in the context of other clinical criteria. The referring clinician is responsible for counseling before and after the test including the provision of advice regarding the need for additional genetic testing. Other diagnostic tests may still be necessary.

SUPPLEMENTARY INFORMATION

Disease (Gene)	Results
Fragile X (FMR1)	Allele 1: 22 repeats Allele 2: 30 repeats

REFERENCES

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Approved by:

Approved by:

Date of report (DD/MM/YYYY):

REPORT EXAMPLE

Table 1 - Genes tested by the Rodinia Infertility Panel

FEMALE INFERTILITY PANEL				
AIRE	EIF2B3	GALT	IRS2	PROKR2
ANOS1	FEZF1	GDF9	KISS1	PSMC3IP
BMP15	FGF17	GNAS	KISS1R	SEMA3A
CAPN10	FGF8	GNRH1	LHB	SPRY4
CHD7	FGFR1	GNRHR	LHCGR	STAG3
CYP11A1	FIGLA	HESX1	NOBOX	TAC3
CYP17A1	FLRT3	HS6ST1	NR5A1	TACR3
CYP19A1	FMR1	IL17RD	NSMF	THADA
DENND1A	FOXL2	INS	POF1B	WDR11
DUSP6	FSHB	INSR	POLG	WT1
EIF2B2	FSHR	IRS1	PROK2	ZP1

MALE INFERTILITY PANEL				
ANOS1	DUSP6	FSHR	LHB	SPRY4
AR	FEZF1	GNRH1	LHCGR	SRD5A1
AURKC	FGF17	GNRHR	NR5A1	SRY
CATSPER1	FGF8	HESX1	NSMF	TAC3
CFTR	FGFR1	HS6ST1	PRM1	TACR3
CHD7	FLRT3	IL17RD	PROK2	USP26
DAZL	FMR1	KISS1	PROKR2	USP9Y
DDX25	FSHB	KISS1R	SEMA3A	WDR11

REPORT EXAMPLE

THROMBOPHILIA AND NAIT PANEL

Disorder / Common name	Gene	Variant	Alternative nomenclature
Factor V Leiden	F5	NM_000130.4(F5):c.1601G>A (p.Arg534Gln)	G1691A F5,ARG506GLN R506Q Factor V Leiden
Factor V R2	F5	NM_000130.4(F5):c.3980A>G (p.His1327Arg)	FV R2 H1299R A4070G R2 allele
Factor XIII	F13A1	NM_000129.3(F13A1):c.103G>T (p.Val35Leu)	p.Val34Leu F13A1; VAL34LEU; V34L
HPA-1	ITGB3	NM_000212.2(ITGB3):c.176T>C (p.Leu59Pro)	L33P
HPA-2	GP1BA	NM_000173.7(GP1BA):c.482C>T (p.Thr161Met)	rs6065
HPA-3	ITGA2B	NM_000419.5(ITGA2B):c.2621T>G (p.Ile874Ser)	I843S
HPA-4	ITGB3	NM_000212.2(ITGB3):c.506G>A (p.Arg169Gln)	R143Q
HPA-5	ITGA2	NM_002203.4(ITGA2):c.1600G>A (p.Glu534Lys)	Not available
HPA-6	ITGB3	NM_000212.2(ITGB3):c.1544G>A (p.Arg515Gln)	R489Q
PAI-1 4G/5G	SERPINE1	NM_000602.5(SERPINE1):c.-820G[(4_5)]	4G/5G
MTHFR	MTHFR	NM_005957.5(MTHFR):c.665C>T (p.Ala222Val)	C677T; MTHFR; 677C-T; ALA222VAL (rs1801133)
MTHFR	MTHFR	NM_005957.4(MTHFR):c.1286A>C (p.Glu429Ala)	MTHFR; 1298A-C; GLU429ALA (rs1801131)
ACE (I/D)	ACE	NM_000789.3(ACE):c.2306-117_2306-116insAF118569.1: g.14094_14382	ACE/ID polymorphism INS/DEL (rs1799752)
Apo B	APOB	NM_000384.3(APOB):c.10580G>A (p.Arg3527Gln)	R3500Q 9775G>A
Apo E	APOE	NM_000041.2(APOE):c.526C>T (p.Arg176Cys)	R158C R148C
Apo E	APOE	NM_000041.4(APOE):c.388T>C (p.Cys130Arg)	C112R ApoE4
MTR	MTR	NM_000254.2(MTR):c.2756A>G (p.Asp919Gly)	p.D919G:GAC>GGC 2756A-G
MTRR	MTRR	NM_002454.3(MTRR):c.66A>G (p.Ile22Met)	p.I49M:ATA>ATG
AGT	AGT	NM_000029.4(AGT):c.803T>C (p.Met268Thr)	M235T NM_000029.3:c.803T>C
AGTR1	AGTR1	NM_031850.3(AGTR1):c.*86A>C	A1166C
GSTP1	GSTP1	NM_000852.4(GSTP1):c.313A>G (p.Ile105Val)	rs1695 GSTP1*B
Prothrombin	F2	NM_000506.5(F2):c.*97G>A	F2 rs1799963 20210G-A G20210A