NIPD GENETICS
A MEDICOVER COMPANY

Rodinia®
infertility test

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	LAB NUMBER	DATE OF COLLECTION	DATE RECEIVED
XXXX	XXXX	XX/XX/XXXX	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**	[redacted]
F5 *(Factor V R2)*	H1299R	NM_000130.4(F5):c.3980A>G (p.His1327Arg)	**Heterozygous**	[redacted]

				[illegible]
F13A1 *(Factor XIII)*	V34L	NM_000129.3(F13A1):c.103G>T (p.Val35Leu)	**Homozygous, Normal Genotype**	[illegible]
ITGB3 *(HPA-1)*	L33P	NM_000212.2(ITGB3):c.176T>C (p.Leu59Pro)	**a/a**	[illegible]
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**	[illegible]
MTHFR	C677T	NM_005957.5(MTHFR):c.665C>T (p.Ala222Val)	**Heterozygous**	[illegible]

MTHFR	A1298C	NM_005957.4(MTHFR):c.1286A>C (p.Glu429Ala)	**Homozygous, Normal Genotype**	[redacted]
ACE *(ACE (I/D))*	ACE/ID polymorphism	NM_000789.3(ACE):c.2306-117_2306-116insAF118569.1:g.14094_14382	**D/D**	[redacted]
APOB	R3500Q	NM_000384.3(APOB):c.10580G>A (p.Arg3527Gln)	**Homozygous, Normal Genotype**	[redacted]
APOE	R158C	NM_000041.2(APOE):c.526C>T (p.Arg176Cys)	**E3/E3**	[redacted]
APOE	C112R	NM_000041.4(APOE):c.388T>C (p.Cys130Arg)		

				[illegible]
MTR		NM_000254.2(MTR):c.2756A>G (p.Asp919Gly)	**Heterozygous**	[illegible]
MTRR	p.I49M:ATA>ATG	NM_002454.3(MTRR):c.66A>G (p.Ile22Met)	**Homozygous for mutation**	
AGT	M235T	NM_000029.4(AGT):c.803T>C (p.Met268Thr)	**Heterozygous**	[illegible]
AGTR1	A1166C	NM_031850.3(AGTR1):c.*86A>C	**Homozygous for mutation**	[illegible]
GSTP1	GSTP1*B	NM_000852.4(GSTP1):c.313A>G (p.Ile105Val)	**Homozygous, Normal Genotype**	[illegible]

				were at 1.7-fold increased HF risk than G1TP1.4a/b carriers. G1TP1 polymorphic variants may determine individual susceptibility to oxidative stress, inflammation, and endothelial dysfunction in HF [1].
F2 *Prothrombin*	G20210A	NM_000506.5(F2):c.*97G>A	**Homozygous, Normal Genotype**	F2 c.*97G>A (also known as c.20210G>A or G20210A) has been associated with increased risk for venous thromboembolism. This variant has been observed in multiple ethnic backgrounds. A recent meta-analysis have reported an odds ratio of 1.3-6.2 for developing venous thromboembolism. The polymorphism in heterozygous prothrombin G20210, heterozygous Factor V Leiden H1299R, PAI-1 4G/5G, and MTR A-2756G have been related to recurrent pregnancy loss [2].

METHODOLOGY/LIMITATIONS

Rodinia is a Laboratory Developed Test (LDT) from NIPD Genetics Public Company Ltd for infertility testing. Genomic deoxyribonucleic acid (DNA) is extracted using a standardized automated protocol and subjected to mechanical fragmentation prior to DNA library preparation. DNA enrichment for the genomic regions 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 pipelines. Rodinia can be used for the identification of single nucleotide variants, small insertions and deletions (≤50bp) and copy number variations (CNVs). Variants are classified according to the criteria set by the American College of Medical Genetics and Genomics [3]. Classification and interpretation of variants is performed using the Varsome Clinical platform and is according to published knowledge at the time of testing. Variants which are classified as pathogenic and 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 carrier status in recessive conditions will not be reported. Genetic counseling for the clinical interpretation and significance of the results is recommended. A 'no clinically significant findings' indicates the absence of an inherited/de novo genetic cause 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. The results that are associated with haemophilia and MAT are listed in a separate table when opted.

The test aims to detect coding 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, sequence changes (SNVs and INDELs) in the promoter and other non-coding regions are not covered by this assay. Certain sequence changes (SNVs and INDELs) in non-coding regions of selected genes that are of clinical significance are also included in the analysis. 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, rearrangements, polyploidy and epigenetic effects are not covered by this test. Certain sequence changes (SNVs 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 can also detect CNVs up to a few exons level with lower sensitivity of the genes tested. All positive CNVs are confirmed with an orthogonal method. The test cannot detect CNVs up to a single or a few exons 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 is reported but does not exclude the possibility of a disease-associated syndrome. Certain chromosomal numerical and structural abnormalities (aneuploidies, copy number changes ≥10Mb, and balanced ≥5Mb) can be detected by Rodinia test. Although the test is highly accurate there is still a possibility for false positive and false negative results.

Chromosome Y microdeletions and Fragile X syndrome are performed using methodologies described below:

Chromosome Y microdeletions: Analysis of the azoospermia factor (AZF) regions on the Y chromosome, which is associated with spermatogenetic 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 aberrations, the possibility remains that biological changes in that gene or 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 fluorescence labeled primer and sized by capillary gel electrophoresis. The interpretation is based on the following ranges of repeat sequences: Negative: <44 repeats, Intermediate: 45-54 repeats, Premutation: 55-200 repeats, Mutated: >200 repeats. Southern blot is not performed, therefore, repeat expansion may not be able to detect exact 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 transfusion, bone marrow transplantation or other rare molecular events.

The Rodinia infertility test development and performance evaluation was carried out by NIPD Genetics Public Company Limited, which is regulated under the Clinical Laboratory Improvement Act of 1990 (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 premarket FDA review.

ADDITIONAL TECHNICAL SPECIFICATIONS

INS, HBB/11R: Deletion/duplication analysis is not performed for these genes

ADDITIONAL INFORMATION / DISCLOSURE

Validation studies are carried out by NIPD Genetics 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 biological limitations, including but not limited to: mislabeled samples, inaccurate reporting of clinical/medical information, rare technical errors, or other rare events. Some undetected genetic changes could be disease-related and are not tested by Rodinia. Genetic testing is an important part of the diagnostic process. However, genetic tests may not always provide a definitive answer. In some cases, testing may not identify a genetic variant even though one exists. This may be due to limitations in 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 referral 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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13. Richards, S. *et al.* Standards and guidelines for the interpretation of sequence variants: A joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet. Med.* (2015) doi:10.1038/gim.2015.30.

Approved by:

Approved by:

Date of report (DD/MM/YYYY):

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

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