PATIENT INFORMATION	REFERRAL INFORMATION
NAME	CLINIC NAME
PATIENT GENETIC SEX **MALE**	CLINIC ID
PATIENT ETHNICITY	CLINIC EMAIL
DATE OF BIRTH **02/03/1989**	REFERRING HEALTHCARE PROVIDER

TEST INDICATIONS
MEDICAL HISTORY

SAMPLE INFORMATION			
ORDER NUMBER	LAB NUMBER	DATE OF COLLECTION **01/09/2023**	DATE RECEIVED **05/09/2023**
TEST PERFORMED	**Rodinia** **Male Infertility panel**		

For a full list of evaluated diseases and genes tested please refer to: **bit.ly/RodiniaTest**

TEST RESULTS

Clinically significant variant(s) detected

NUMERICAL / STRUCTURAL ABNORMALITIES RESULTS

No numerical or structural abnormalities detected

GENOMIC FINDINGS

GENE	VARIANT	ZYGOSITY	INHERITANCE	VARIANT CLASSIFICATION
GNRHR	NM_000406.3:c.785G>A (p.Arg262Gln)	Heterozygous	Autosomal Recessive	Pathogenic

INTERPRETATION

Variant Detected
GNRHR NM_000406.3:c.785G>A(p.Arg262Gln)

Variant Summary
A heterozygous c.785G>A variant was detected in exon 3 of the GNRHR gene (NM_000406.3). This is a missense variant which changes the amino acid arginine to glutamine at position 262 of the protein sequence. This amino acid change is conservative in terms of the physicochemical properties of these amino acids. This variant is found in a mutational hot-spot of the protein with low benign variation. This variant is present in the GnomAD population databases in a frequency less than what is expected for an autosomal recessive allele[1]. Computational evidence supports a strong benign effect of this variant on the gene or gene product. This variant has been submitted in the ClinVar database and classified as pathogenic/ likely pathogenic (Variation ID: 16024)[2]. In addition, this variant has been submitted in the LOVD database and classified as pathogenic[3]. Also, an alternative variant at the same position, p.Arg262Trp, has been classified as likely pathogenic by ClinVar[2] and confirmed using ACMG criteria[4]. Based on the information outlined above and according to the ACMG guidelines this variant is classified as pathogenic[4].

Gene Information and Significance
Gonadotropin-releasing hormone receptor (GNRHR) gene encodes the receptor for type 1 gonadotropin-releasing hormone[5]. This receptor is a member of the G protein-coupled receptor (GPCR) family[5,6]. It is expressed on the surface of pituitary gonadotrope cells as well as lymphocytes, breast and ovary[5]. Following binding of gonadotropin-releasing hormone, the receptor associates with G-proteins that activate a phosphatidylinositol-calcium second messenger system[5]. Activation of the receptor ultimately causes the release of gonadotropic luteinizing hormone (LH) and follicle stimulating hormone (FSH)[5] which in turn regulate gametogenic and hormonal functions on gonads[6]. Defects in this gene are a cause of hypogonadotropic hypogonadism (HH)[5]. Mutations in GNRHR gene, sometimes in association with mutations in another gene, cause hypogonadotropic hypogonadism-7 with or without anosmia (HH7)[7,8]. According to the literature, hypogonadotropic hypogonadism-7 with or without anosmia (HH7) is caused by homozygous or compound heterozygous mutations in GNRHR gene, however, increasing numbers of patients with heterozygous variants in GNRHR are being reported[8,9]. Congenital idiopathic hypogonadotropic hypogonadism (IHH) or HH7 is a disorder characterized by absent or incomplete sexual maturation by the age of 18 years, in conjunction with low levels of circulating gonadotropins and testosterone and no other abnormalities of the hypothalamic-pituitary axis[6]. Other associated nonreproductive phenotypes, such as anosmia, cleft palate, and sensorineural hearing loss, occur with variable frequency[10]. In the presence of anosmia, idiopathic hypogonadotropic hypogonadism is called Kallmann syndrome (KS), whereas without anosmia is called normosmic idiopathic hypogonadotropic hypogonadism (nIHH)[6].

RECOMMENDATIONS

This individual is predicted to be a heterozygous carrier for the c.785G>A variant in the GNRHR gene. This interpretation is based on the clinical information provided and the current understanding of the molecular genetics of this condition. The result should be evaluated in the context of all clinical findings and patient history. This individual has a 50% chance of passing on the mutation to his/her offspring. Genetic testing is recommended to this individual's partner and family members. Genetic counselling is recommended for all individuals undergoing genetic testing. Clinical correlation with other data and tests is recommended.

SUPPLEMENTARY INFORMATION

DISEASE (GENE)	RESULTS
Fragile X syndrome (FMR1)	Allele 1: 33 repeats Allele 2: -

ANALYSIS STATISTICS

TARGETED NUCLEOTIDES COVERED	≥ 10x : **97.5%** ≥ 20x : **96.5%**

METHODOLOGY / LIMITATIONS

Rodinia is a Laboratory Developed Test (LTD) from Medicover Genetics for infertility testing. Genomic deoxyribonucleic acid (gDNA) is extracted using a standardized methodology 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 (≤30bp) and copy number variations (CNVs). Variants are classified according to the criteria set by the American College of Medical Genetics and Genomics [4]. 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 counselling for the clinical interpretation and significance of the results is recommended. A 'no clinically significant variant detected' result indicates the absence of an inherited/de novo variant and reduces but does not guarantee that the individual does not have a genetic cause for his/her condition. A 'clinically significant variant detected' result indicates that a genetic change has been identified and that the individual will likely develop the condition. The results that are associated with Thrombophilia and NAIT are listed in a separate table when opt-in. Thrombophilia and NAIT stand-alone panel tests and reports only the selected variants listed in the table.
The test aims to detect all variants relevant to the tested genes, targeting all coding exons, of MANE or/and Canonical transcripts and 10 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 one 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 high/low GC-content may not be detected. Copy Number Variations (CNVs) are calculated using high quality, de-duplicated and uniquely aligned sequencing reads. CNVs are detected for a subset of the targeted regions using a depth of sequencing coverage approach by applying GC-content normalization. Genomic regions are called as variants if their normalized depth of coverage deviates significantly from the expected normalized coverage which is estimated from a set of reference clinical samples. The test is designed to detect CNVs at the gene level for the genes tested, unless otherwise noted, with high sensitivity and specificity. The test can also detect CNVs down to a few exons level with lower sensitivity of the genes tested. All positive CNVs are confirmed using an orthogonal method. The test cannot detect CNVs at genomic regions with either low mappability or containing repeats, pseudogenes and high/low GC-content. Detection of CNVs using NGS has lower sensitivity/specificity than orthogonal quantification methods, therefore the absence of reported CNVs does not guarantee the absence of CNVs. The lack of disease-causing variants in the targeted genes diminishes but does not exclude the possibility of a disease associated syndrome. Sex chromosomal numerical and structural abnormalities (aneuploidies, copy number changes ≥10Mb, and mosaicism ≥15%) can be detected by Rodinia test. Although the test is highly accurate there is still a possibility for false positive or false negative results.

Chromosome Y microdeletions and Fragile X syndrome are performed using methodologies described below:
Chromosome Y microdeletions: Analysis of the azoospermia factors (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, Full Mutation: >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 Inferility test development and performance evaluation was carried out by Medicover Genetics, which is regulated under the Clinical Laboratory Improvement Act of 1998 (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

Technical Specifications may vary according to the selected test.

Genomic regions not covered by the female infertility panel: NM_001282717.2 (STAG3): exon 7; NM_001004311.3 (FIGLA): exons 1 and 4; NM_004807.3 (HS6ST1): exon 2; NM_001352964.2 (DENND1A): exons 1, 20 and 22; NM_000208.4 (INSR): exon 1; NM_033163.5 (FGF8): exon 1; NM_000894.3 (LHB): exon 1; NM_001130969.3 (NSMF): exon 1; NM_001126128.2 (PROK2): exon 1.

Genomic regions not covered by the male infertility panel: NM_004807.3 (HS6ST1): exon 2; NM_001351.4 (DAZL): exons 7, 9, 10 and 11; NM_033163.5 (FGF8): exon 1; NM_000894.3 (LHB): exon 1; NM_001130969.3 (NSMF): exon 1; NM_001126128.2 (PROK2): exon 1.

ADDITIONAL INFORMATION / DISCLOSURE

Validation studies are carried out by Medicover Genetics 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, rare technical errors, or other rare events. These include rare genetic variants, mosaicism, blood transfusion, bone marrow transplantation or other rare molecular 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 give 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 counselling before and after the test including the provision of advice regarding the need for additional genetic testing. Other diagnostic tests may still be necessary.

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Approved by:

Approved by: Laboratory Director

Date of report (DD/MM/YYYY):

For a full list of evaluated diseases and genes tested please refer to: **bit.ly/RodiniaTest**

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