Male Fertility and Polymorphism Panel

Patient name	:	**XY**	**PIN**	:	**XXX**
Gender/ Age	:	**XXX**	**Sample number**	:	**XXX**
Hospital/Clinic	:	**XXX**	**Sample collection date**	:	**XXX**
Specimen	:	**Peripheral blood**	**Sample receipt date**	:	**XXX**
			Report date	:	**XXX**

CLINICAL HISTORY

Mr. XY and Mrs. XX are a non-consanguineous couple presented with a history of pregnancy losses. Mr. XY has been evaluated for male infertility single nucleotide variation and single nucleotide polymorphism.

RESULTS

MALE FERTILITY SINGLE NUCLEOTIDE VARIATION

No pathogenic or likely pathogenic variant causative of the reported phenotype was detected

*Genetic test results are based on the recommendation of American college of Medical Genetics [1-3].
No other variant that warrants to be reported for the given clinical indication was identified.

MALE POLYMORPHISM ANALYSIS			
GENE NAME	**VARIANT**	**GENOTYPE**	**INTERPRETATION**
CATSPER1	*CATSPER1*:c.1954G>A	AA	*CATSPER1* is a sperm specific ion channel gene essential for sperm hyperactivated motility and male fertility. Homozygous variations of *CATSPER1* are associated with Spermatogenic Failure 7 (OMIM#612997). Phenotype includes asthenozoospermia with non-motile sperm or sperm motility below the normal threshold, low sperm count, increased abnormally structured spermatozoa and reduced semen volume. ICSI is an effective treatment in patients with *CATSPER* mutations as it is compatible with normal embryonic development [10 -13].

FSHR	*FSHR:* c.2039G>A	TT	The influence of *FSHR* gene variants on male gonadic function and reproductive parameters is significant but with wide variability of presentation. FSH action on Sertoli cells is altered by *FSHR* gene variants resulting in azoospermia, oligospermia and reduced motility due to disturbed feedback loop within the hypothalamic pituitary–testis axis. High DNA fragmentation is also reported. The genotype reported is shown to have 3-fold increased risk for impaired spermatogenesis on Indian population [14-16].
CFAP44	*CFAP44:* c.2234C>T	GA	Cilia And Flagella Associated Protein 44 is required for formation of sperm flagella and normal sperm motility. Variations in *CFAP* genes may lead to sperm flagellar malformations, which include absent, short, coiled, bent, and/or irregular-caliber flagella and impair sperm motility and function. Spermatogenic Failure 20 (OMIM#617593). Intracytoplasmic sperm injection is an effective treatment in patients with *CFAP44* mutations as it is compatible with normal embryonic development [17-18].
	CFAP44: c.852G>T	CA	

MALE INFERTILITY GENES - 755 GENES

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, KISS1R, CYP21A2, CYP17A1, CYP11B1, CYP11B2, POR, SRD5A2, PSMC3IP, STAG3, MEIOB, FANCM, SETX, E2F1, MEI1, PIWIL1, NPAS2, TEX11, TEX14, NANOS2, WNK3, SPO11, CCDC155, UTP14C, DNAH6, FSHB, WT1, DAZ1, TSPYL1, DZIP1, FAM47C, SPAG17, TDRD6, AKAP9, CCDC146, TERB1, CEP131, ASZ1, CFAP44, DDX25, DNAAF2, DNAH1, DNAH5, ELMO1, ESR2, HIPK4, HORMAD1, MAGEE2, NANOS1, ODF4, PGK2, PIWIL2, ROS1, SLC26A8, SPATA3, SPIDR, STRA8, SYCP2, TBCCD1, TTC21A, TTLL9, XRCC2, ZSWIM7, TDRD9, SYCP3, NLRP14, CST1, DNAH7, NLGN4Y, PPP1R36, TCEANC, ZNF541, SIRPG, CEP250, TDP1, SYCE1, SOHLH1, SPATA22, HSF2, C14orf39, SHOC1, CSTF2T, HFM1, SUN1, TEX12, MSH5, PICK1, TDRD7, DMC1, RAD51AP2, FBXO43, M1AP, SCAPER, PNLDC1, MSH4, CEP70, ABCB11, ACADS, AMH, ATM, CBS, CLN3, CTSD, CYP19A1, ERCC2, HSD17B4, HSD3B2, MEFV, MTRR, NPHP1, PANK2, PEX10, POLG, XPC, BRDT, DMRT1, GNAS, KLHL10, LEP, LEPR, NR3C1, PRDM9, SOX3, NR0B1, RPL10L, HYDIN, MMRN1, SGO2, DMRTA2, MOSPD2, RIOK2, STAG2, ZMYND15, SYCP1, MLH3, CYP1B1, DHCR7, GAA, GALNT3, GH1, HMOX1, MTHFR, NPC2, NTRK1, WAS, ACE, SHBG, TAF4B, DNAH17, ADCY10, AKAP4, CATSPER2, CATSPER3, CATSPER4, PLA2G6, TEKT2, GALNTL5, POLR2C, FSIP2, DNALI1, ARMC12, TCTE1, STK33, IQUB, KATNAL2, CCDC34, AKAP3, SLC9C1, DNHD1, CCIN, SEPTIN4, AAAS, BRIP1, CHRNG, CIITA, CLN6, EDA, EIF2B5, FANCC, FKTN, GJB6, GP1BA, GUCY2D, HBA1, HMGCL, HSD17B3, IL2RG, LAMB3, LIFR, MYO15A, NEU1, OAT, PNPO, POMGNT1, RAG2, RDH12, RLBP1, SAMD9, SLC19A2, STRC, TAT, TCIRG1, TYRP1, ZFYVE26, MAP3K1, PLCZ1, CATSPERE, ACTL7A, KCNU1, ACR, WEE2, ACTL9, NBN, SUN5, PMFBP1, BSCL2, SPATA20, ARMC2, PRSS55, TTC29, WDR12, RNF220, ABCA12, ABCA4, ABCB4, ACAD9, ACADM, ACADSB, ACADVL, AGL, AGPS, AIRE, ALDH3A2, ALDOB, ALG6, ALMS1, AMHR2, AP1S1, AQP2, ARSB, ASNS, ASS1, ATP6V1B1, ATP7A, ATP7B, BBS12, BBS2, BBS4, BCKDHB, BCS1L, BTD, CANT1, CAPN3, CASQ2, CEP290, CHRNE, CLN8, CNGB3, COL11A2, COL4A3, COL7A1, CPS1, CPT1A, CPT2, CRB1, CTNS, CTSC, CTSK, CYBA, CYBB, CYP27B1, DCLRE1C, DDB2, DKC1, DLD, DMD, EMD, ERCC4, ERCC5, ERCC6, ERCC8, ETFB, ETFDH, ETHE1, EXOSC3, FAH, FAM161A, FANCG, FH, GALC, GALE, GALNS, GBE1, GCDH, GCH1, GFM1, GJB1, GJB3, GLA, GLDC, GLE1, GNE, GNS, GP1BB, GP9, GRHPR, GUSB, HADHA, HADHB, HAX1, HBA2, HBB, HEXA, HEXB, HGD, HPS3, HPS4, HYLS1,

IDS, ITGB3, IVD, LAMC2, LDLR, LIPH, LOXHD1, LPL, LRPPRC, MCCC1, MCCC2, MECP2, MFSD8, MKKS, MLC1, MLYCD, MMAB, MMADHC, MPI, MPL, MRE11, MTM1, MTTP, NDUFAF5, NDUFS4, NDUFS6, NEB, NLRP7, NPC1, NR2E3, OCRL, OPA3, PAH, PC, PCCA, PCCB, PDHA1, PDHB, PEPD, PEX1, PEX12, PEX2, PEX7, PFKM, PHGDH, PIGN, POLH, PPT1, PREPL, PRPS1, PSAP, PTS, PUS1, RAB23, RAG1, RAPSN, RARS2, RTEL1, SACS, SAMHD1, SBDS, SEPSECS, SERPINA1, SGCB, SGCD, SGSH, SLC12A6, SLC17A5, SLC22A5, SLC25A15, SLC26A2, SLC26A3, SLC26A4, SLC35A3, SLC37A4, SLC3A1, SLC45A2, SLC7A9, SMARCAL1, SMPD1, STAR, SUCLA2, SURF1, TECPR2, TGM1, TPO, TPP1, TRIM32, TRIM37, TSFM, TSHB, TTC37, TTN, TTPA, TYR, UPB1, USH1C, VPS13A, VPS45, VPS53, VRK1, WNT10A, XPA, AXL, BBS5, CYP11A1, GATA4, LHX3, POU1F1, SLC22A2, SOX9, TFPI, DPY19L2, SPATA16, ZPBP, CCDC62, DNAJB13, ARL2BP, DNAL1, DNAAF11, DNAH9, CFAP300, GAS8, DNAAF1, ZMYND10, DNAAF4, RPGR, DNAAF5, DNAH11, ODAD2(ARMC4), NME8, DNAI1, CCDC39, DNAAF3, DNAI2, RSPH1, RSPH4A, RSPH9, TTC12, CCDC40, CEP164, QRICH2, CFAP70, CFAP43, CFAP69, CFAP47, SPEF2, ADAD2, CEP78, CT55, DNAH8, CFAP57, CFAP61, DRC1, CFAP54, CFAP58, CFAP74, MAATS1, WDR19, DNAH2, BRWD1, CFAP206, DNAH10, IFT74, ACAT1, ADGRG2, FANCA, ATRX, FTHL17, CBX2, DHH, ABCC6, ABCC8, ABCD1, ACOX1, ACSF3, ADAMTS2, ADGRG1, AGXT, ALPL, ASL, ATP8B1, BBS1, BCHE, BCKDHA, BLM, BTK, CC2D1A, CDH23, COL4A4, DOK7, DPYD, DYSF, EDAR, EIF2AK3, ETFA, EVC, EVC2, EYS, F11, F2, F8, FKRP, G6PD, GALK1, GALT, GAMT, GBA, GDF5, GLB1, GNPTG, GORAB, HGSNAT, HLCS, HOGA1, HPD, HPS1, IDUA, LAMA2, LAMA3, LCA5, LIPA, MAN2B1, MAT1A, MCOLN1, MMAA, MMACHC, MPV17, MYO7A, NAGLU, NDRG1, NPHS1, PCDH15, PET100, PKHD1, PYGM, RPE65, RPGRIP1L, SGCA, SLC12A3, SLC25A13, SLC25A20, SLC39A4, SLC4A11, SLC6A8, SLC7A7, SUMF1, TFR2, TH, TMC1, TREX1, TRMU, TSHR, TYMP, UGT1A1, USH2A, VPS13B, VWF, AKR1C4, CAPN10, DMRT2, FGFR2, INSL3, LHX4, PCSK1, SRA1, TTC8, WWOX, ABCA3, AFF2, AGA, AHI1, ANO10, ARSA, ARX, ASPA, CC2D2A, CCDC88C, CLCN1, CLRN1, CYP27A1, DHDDS, DYNC2H1, ELP1, F9, FMO3, FXN, G6PC1(G6PC), GJB2, GNPTAB, GRIP1, L1CAM, LRP2, MCPH1, MID1, MMUT, MVK, NAGA, OCA2, OTC, PLP1, PMM2, PRF1, RNASEH2B, RS1, SCO2, SLC19A3, SMN1, TF, TMEM216, TNXB, APC, MYH11, ACTA2, TMEM43, DSP, PKP2, DSG2, DSC2, BRCA1, BRCA2, SCN5A, RYR2, FLNC, LMNA, MYBPC3, COL3A1, APOB, MYH7, TPM1, PRKAG2, TNNI3, MYL3, MYL2, ACTC1, RET, PALB2, HFE, ENG, ACVRL1, MAX, TMEM127, PCSK9, BMPR1A, SMAD4, TNNT2, TP53, TGFBR1, TGFBR2, SMAD3, TRDN, KCNQ1, KCNH2, MLH1, MSH2, MSH6, PMS2, RYR1, CACNA1S, FBN1, HNF1A, MEN1, MUTYH, NF2, SDHD, SDHAF2, SDHC, SDHB, STK11, PTEN, RB1, TSC1, TSC2, VHL, CCDC141, POLR3A, TUBB3, CHD4, RAB3GAP2, SLC29A3, DCAF17, POLA1, POLR3B, POLD1, BBS9, MC2R, EIF2S3, PHF6, RAB3GAP1, PNPLA6, SCLT1, ARL6, SOX2, PROP1, SEMA3A, PROK, SEMA3E, NDNF, NOS1, SIN3A, SOX10, WDR11, TCF12

MALE POLYMORPHISM RSID

rs2292596, rs808119, rs1800477, rs10783071, rs3088232, rs12676, rs4680, rs1048943, rs700519, rs1052763, rs2302075, rs2274911, rs553509, rs3816183, rs605059, rs2227956, rs5498, rs5911, rs1127354, rs631357, rs3764147, rs34349826, rs175080, rs26279, rs2075789, rs1801394, rs1799930, rs1799983, rs1110061, rs1052133, rs1052133, rs11204546, rs57607909, rs662, rs2227973, rs1390938, rs1051266, rs4880, rs12623569, rs523349, rs1877031, rs1713449, rs6525433, rs1800471, rs8103849, rs25487, rs222859, rs2369679, rs10747493, rs397508617, rs2228612, rs13181, rs1799793, rs6166, rs6165, rs25640, rs11205, rs2293275, rs1801131, rs1801133, rs162036, rs1800566, rs11531577, rs10246939, rs1042522, rs3743044, rs1695, rs1801106, rs5918, rs1805388, rs1805087, rs1799931, rs2297518, rs1106042, rs11703684, rs1057868, rs6259, rs2270641, rs323344, rs323345, rs323346, rs323347, rs1805388, rs2272837, rs4997052, rs7946, rs699664, rs1042838, rs1800595, rs3740753, rs5764698, rs11573, rs1056836, rs2003783, rs17632542, rs4844247, rs2066853, rs429358, rs6025, rs7412, rs5985, rs17855750, rs34605051, rs2241057, rs769423, rs1136410, rs140506267**,** rs3814747, rs58988763, rs16845107, , rs17747401, rs1800100, rs1131692251, rs1131692250, rs1131692234, rs779490893, rs113993960, rs11135484, rs10249788, rs4880

Methodology: Single Nucleotide Variation - Single Nucleotide Polymorphism

SNV analysis: DNA extracted from the blood, was used to perform targeted gene capture using a custom capture kit. The targeted libraries were sequenced to a targeted depth of 80 to 100X using GenoLab M sequencing platform. This kit has deep exonic coverage of all the coding regions including the difficult to cover regions. The sequences obtained are aligned to human reference genome (GRCh38.p13) using Sentieon aligner and analyzed using Sentieon for removing duplicates, recalibration and re-alignment of indels. Sentieon DNAscope has been used to call the variants. Detected variants were annotated and filtered using the VarSeq software with the workflow implementing the ACMG guidelines for variant classification. The variants were annotated using 1000 genomes (V2), gnomAD (3.1.2,2.1.1), ClinVar, OMIM, dbSNP, NCBI RefSeq Genes. *In-silico* predictions of the variant was carried out using VS-SIFT, VS-PolyPhen2, PhyloP, GERP++, GeneSplicer, MaxEntScan, NNSplice, PWM Splice Predictor. Only non-synonymous and splice site variants found in the coding regions were used for clinical interpretation. Silent variations that do not result in any change in amino acid in the coding region are not reported.

SNP analysis: Variant analysis and interpretation is done using VarSeq Software. Extensive scientific literature, Information from variant analysis and disease specific databases, population specific research are used to interpret and recommend. All results are finally approved by medical geneticists.

Disclaimer

- The classification of variants of unknown significance can change over time. Anderson Diagnostics and Labs cannot be held responsible for it.
- Intronic variants, UTR, Promoter region variants and CNV are not assessed using this assay.
- Certain genes may not be covered completely, and few mutations could be missed. Variants not detected by this assay may impact the phenotype.
- The variations have not been validated by Sanger sequencing.
- The above findings and result interpretation was done based on the clinical indication provided at the time of reporting.
- It is also possible that a pathogenic variant is present in a gene that was not selected for analysis and/or interpretation in cases where insufficient phenotypic information is available.
- Genes with pseudogenes, paralog genes and genes with low complexity may have decreased sensitivity and specificity of variant detection and interpretation due to inability of the data and analysis tools to unambiguously determine the origin of the sequence data in such regions.
- Incidental or secondary findings that meet the ACMG guidelines can be given upon request [3].
- Result interpretation was done based on the literature evidence available at the time of reporting. The clinical significance of the polymorphic variants tested can change over time and Anderson Diagnostics & Labs cannot be held responsible for this.
- This is not a diagnostic test and so not to be considered as diagnosis of any disease. This test is meant only for understanding the polymorphism at a given position and its association with various clinical parameters.

References

1. 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. Genetics in medicine: official journal of the American College of Medical Genetics. 17.5 (2015): 405-424.
2. Amberger J, Bocchini CA, Scott AF, Hamosh A. McKusick's Online Mendelian Inheritance in Man (OMIM). Nucleic Acids Res. 2009 Jan;37(Database issue):D793-6. doi: 10.1093/nar/gkn665. Epub 2008 Oct 8.
3. Kalia S.S. et al., Recommendations for reporting of secondary findings in clinical exome and genome sequencing, 2016 update (ACMG SF v2.0): a policy statement of the American College of Medical Genetics and Genomics. Genet Med., 19(2):249-255, 2017.
4. Kujovich, J. Factor V Leiden thrombophilia. Genet Med 13, 1–16 (2011).
5. Whayne TF. Methylenetetrahydrofolate Reductase C677T Polymorphism, Venous Thrombosis, Cardiovascular Risk, and Other Effects. Angiology. 2015;66(5):401-404.
6. Machado M, Neto D, Nunes S, et al. The Importance of *MTHFR* C677T/A1298C Combined Polymorphism in Deep Vein Thrombosis: A Case Report. Cureus 14(9): e29265. (2022).
7. Fawzy, M.S., Toraih, E.A., Aly, N.M. et al. Atherosclerotic and thrombotic genetic and environmental determinants in Egyptian coronary artery disease patients: a pilot study. BMC Cardiovasc Disord 17, 26 (2017).
8. Nagato LC, De Souza Pinhel MA, De Godoy JMP, et al. Association of *ApoE* genetic polymorphisms with proximal deep venous thrombosis. J Thromb Thrombolysis 2012; 33: 116–119.
9. Rastogi P, Kumar N, Ahluwalia J, et al. Thrombophilic risk factors are laterally associated with Apolipoprotein E gene polymorphisms in deep vein thrombosis patients: An Indian study. Phlebology. 2019;34(5):324-335.
10. Avenarius MR, et al. Human male infertility caused by mutations in the *CATSPER1* channel protein. Am J Hum Genet. 2009 Apr;84(4):505-10. doi: 10.1016/j.ajhg.2009.03.004. Epub 2009 Apr 2. PMID: 19344877; PMCID: PMC2667975.
11. Cannarella R, et al. New insights into the genetics of spermatogenic failure: a review of the literature. Hum Genet. 2019 Feb;138(2):125-140. doi: 10.1007/s00439-019-01974-1. Epub 2019 Jan 17. PMID: 30656449.
12. Hildebrand MS, et al. Genetic male infertility and mutation of *CATSPER* ion channels. Eur J Hum Genet. 2010 Nov;18(11):1178-84. doi: 10.1038/ejhg.2010.108. Epub 2010 Jul 21. PMID: 20648059; PMCID: PMC2987470.
13. Shu F, et al. Analysis of the correlation of *CATSPER* single nucleotide polymorphisms (SNPs) with idiopathic asthenospermia. J Assist Reprod Genet. 2015 Nov;32(11):1643-9. doi: 10.1007/s10815-015-0548-5. Epub 2015 Sep 8. PMID: 26354096; PMCID: PMC4651948.
14. Simoni M, et al. Treatment with human, recombinant FSH improves sperm DNA fragmentation in idiopathic infertile men depending on the FSH receptor polymorphism p.N680S: a pharmacogenetic study. Hum Reprod. 2016 Sep;31(9):1960-9. doi: 10.1093/humrep/dew167. Epub 2016 Jun 20. PMID: 27329968.
15. Casamonti, et al. "Short-term FSH treatment and sperm maturation: a prospective study in idiopathic infertile men." Andrology 5.3 (2017): 414-422.

16. Udumudi, et al. Favorable outcome of r-FSH treatment in male with homozygous Ser680ASN variant in *FSHR* gene: a case report demonstrating pharmacogenomic implication in male infertility. Fertility Science and Research 8(2):p 185-187, Jul–Dec 2021. | DOI: 10.4103/fsr.fsr_42_21
17. Alhathal N, et al. A genomics approach to male infertility. Genet Med. 2020 Dec;22(12):1967-1975. doi: 10.1038/s41436-020-0916-0. Epub 2020 Jul 28. PMID: 32719396.
18. Coutton C, et al. Mutations in *CFAP43* and *CFAP44* cause male infertility and flagellum defects in Trypanosoma and human. Nat Commun. 2018 Feb 15;9(1):686. doi: 10.1038/s41467-017-02792-7. PMID: 29449551; PMCID: PMC5814398.

This report has been reviewed and approved by:

Sivasankar.S, Ph.D
Molecular Biologist

Muthukumaran. S, Ph.D
Clinical Bioinformatician

Dr. Parminder Kaur
DM Medical Genetics
Clinical Geneticist

Sachin. D.Honguntikar, Ph.D,
Molecular Geneticist

Dr. G. Suriyakumar
Director