Female Fertility and Polymorphism Panel

Patient name	:	**XXX**	**PIN**	:	**XXXXX**
Gender/ Age	:	**29 Years / Female**	**Sample number**	:	**632403262**
Hospital/ Clinic	:	**XXXX**	**Sample collection date**	:	**18-10-2024**
Specimen	:	**Peripheral blood**	**Sample receipt date**	:	**18-10-2024**
			Report date	:	**15-11-2024**

INDICATION FOR TESTING

Proband, XXX is married non-consanguineous and presented with clinical diagnosis of premature ovarian failure. She has irregular menstrual cycle, USG abdomen was indicative of hypoplastic uterus and left ovary agenesis. MRI Pelvis was indicative of smaller sized uterus age and bilateral smaller sized ovaries. Her *AMH* level is <0.0010ng/ml. Peripheral blood karyotyping is indicative of 46,X,t(X;21)(q22;p11.1). Proband, XXX has been evaluated for pathogenic and polymorphic variations.

RESULTS

FEMALE FERTILITY SINGLE NUCLEOTIDE VARIATION ANALYSIS

Variant of uncertain significance was identified in *BMP15* gene

List of uncertain significant variant identified related to phenotype:

Gene	Region	Variant*	Allele Status	Disease	Classification*	Inheritance pattern
BMP15 (+)	Exon 1	c.226C>T **(p.Arg76Cys)**	Heterozygous	Ovarian dysgenesis 2/ Premature ovarian failure 4 **(OMIM#300510)**	**Uncertain significance**	X linked

*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.

Single Nucleotide Variation - Interpretation

***BMP15*: c.226C>T**

Variant summary: A heterozygous missense variant in exon 1 of the *BMP15* gene (chrX:g.50911009C>T; NM_005448.2, Depth: 113x) that results in the amino acid substitution of Cysteine for Arginine at codon 76 (p.Arg76Cys) was detected.

Population frequency: This variant has minor allele frequency of 0.032% in gnomAD database and has minor allele frequency of 0.1325% in 1000 genomes database.

Clinical and Literature evidence: This variant has been classified as pathogenic in ClinVar database [4]. This variant has been previously reported in patients affected with Premature ovarian failure in heterozygous state [5].

***In-silico* prediction**: The *in-silico* predictions of the variant are damaging by SIFT and PolyPhen-2 (HumDiv). The reference codon is conserved across mammals in PhyloP and GERP++ tools.

OMIM phenotype: Ovarian dysgenesis 2/Premature ovarian failure 4 (OMIM#300510) are caused by mutation in the *BMP15* gene (OMIM*300247). These diseases follow X linked pattern of inheritance [2].

Variant classification: Based on the evidence, this variant has been classified as a variant of uncertain significance. **In this view, clinical correlation and familial segregation analysis are strongly recommended to establish the significance of the finding. If the results do not correlate, additional testing may be considered based on the phenotype observed.**

Additional Variant(s)

List of significant carrier variants identified:

Gene	Region	Variant*	Allele Status	Disease	Classification*	Inheritance pattern	Literature evidence
ABCA4 (-)	Intron 13	c.1937+1G>A **(5' Splice site)**	Heterozygous	Cone-rod dystrophy 3 **(OMIM#604116)** Fundus flavimaculatus / Retinal dystrophy, early-onset severe **(OMIM#248200)**	**Likely pathogenic**	Autosomal Recessive	ClinVar: 99104 PubMed: 24585425

				Retinitis pigmentosa 19 **(OMIM#601718)** Stargardt disease 1 **(OMIM#248200)**			
GJB2 (-)	Exon 2	c.71G>A **(p.Trp24Ter)**	Heterozygous	Deafness, autosomal recessive 1A **(OMIM#220290)**	**Likely pathogenic**	Autosomal Recessive	ClinVar: 17002 Pubmed: 35707775
TH (-)	Exon 11	c.1147G>A **(p.Gly383Arg)**	Heterozygous	Segawa syndrome, recessive **(OMIM#605407)**	**Likely pathogenic**	Autosomal Recessive	ClinVar: 2077702 Pubmed: 19491146
NUP107 (+)	Exon 14	c.1191delT **(p.Val398LeufsTer12)**	Heterozygous	?Ovarian dysgenesis 6 **(OMIM#618078)** Galloway-Mowat syndrome 7 **(OMIM#618348)** Nephrotic syndrome, type 11 **(OMIM#616730)**	**Likely pathogenic**	Autosomal Recessive	-

FEMALE POLYMORPHISM ANALYSIS

List of polymorphic variants (SNP) identified:

GENE NAME	VARIANT	GENOTYPE	INTERPRETATION
#*AMH*	*AMH*: c.1544T>C (p.Val515Ala)	CC	The *AMH* Ala515Val variant may remain common in the population because its increased activity compensates for its reduced secretion [6].

#Genotype – phenotype correlation is strongly recommended.

FEMALE INFERTILITY GENES - 679 GENES

Genes:*PROKR2,ANOS1,FGFR1,CHD7,SEMA3A,CYP17A1,CYP21A2,PATL2,TUBB8,TRIP13,ZP3,CBS,ZP1,ZP2,PADI6,TLE6,KHDC3L,NLRP7,NLRP5,BTG4,CHEK1,WEE2,PANX1,LHX1,WNT4,SHOX,HNF1B,TBX6,WNT9B,TBC1D1,AMH,AMHR2,CLPP,HSD17B4,DNAH5,DNAI1,DNAI2,DNAL1,BRCA1,CFTR,LHCGR,DLX3,FGG,LIG4,COX4I2,AR,CBX2,CYP11A1,CYP19A1,DHH,FGF8,FSHB,HESX1,HSD17B3,LHB,LHX3,LHX4,MAP3K1,NR0B1,NSMF,POU1F1,PROP1,SRD5A2,AAAS,ABCA12,ABCA4,ABCB11,ABCB4,ABCC6,ABCC8,ABCD1,ACAD9,ACADM,ACADS,ACADSB,ACADVL,ACAT1,ACOX1,ACSF3,ADA,ADAMTS2,ADGRG1,AGA,AGL,AGPS,AGXT,AIRE,ALDH3A2,ALDH7A1,ALDOB,ALG6,ALPL,AMT,AP1S1,AQP2,ARG1,ARSA,ARSB,ASL,ASNS,ASPA,ASS1,ATM,ATP6V1B1,ATP7A,ATP7B,ATP8B1,ATRX,BBS1,BBS10,BBS2,BBS4,BBS9,BCHE,BCKDHA,BCKDHB,BCS1L,BLM,BRIP1,BSND,BTD,BTK,CANT1,CAPN3,CASQ2,CC2D1A,CDH23,CEP290,CERKL,CHM,CHRNE,CHRNG,CIITA,CLN3,CLN5,CLN6,CLN8,CLRN1,CNGA3,CNGB3,COL11A2,COL4A3,COL4A4,COL4A5,COL7A1,CPS1,CPT1A,CPT2,CRB1,CTNS,CTSC,CTSD,CTSK,CYBA,CYBB,CYP11B2,CYP1B1,CYP27A1,CYP27B1,DBT,DCLRE1C,DDB2,DHCR7,DHDDS,DKC1,DLD,DMD,DOK7,DPYD,DYSF,EDA,EDAR,EIF2AK3,EMD,ERCC2,ERCC3,ERCC4,ERCC5,ERCC8,ESCO2,ETFA,ETFB,ETFDH,ETHE1,EVC,EVC2,EXOSC3,EYS,F11,F2,F8,F9,FAH,FAM161A,FANCA,FANCC,FANCG,FH,FKRP,FKTN,G6PC,G6PD,GAA,GALC,GALE,GALK1,GALNS,GALNT3,GAMT,GBA,GBE1,GCDH,GCH1,GDF5,GFM1,GH1,GHRHR,GJB1,GJB2,GJB3,GJB6,GLA,GLB1,GLDC,GLE1,GNE,GNPTAB,GNPTG,GNS,GORAB,GP1BA,GP1BB,GP9,GRHPR,GUCY2D,GUSB,HADHA,HADHB,HAX1,HBA1,HBA2,HBB,HEXA,HEXB,HFE,HFE2(HJV),HGD,HGSNAT,HLCS,HMGCL,HMOX1,HOGA1,HPD,HPS1,HPS3,HPS4,HSD3B2,HYLS1,IDS,IDUA,IKBKAP(ELP1),IL2RG,ITGB3,IVD,KCNJ11,LAMA2,LAMA3,LAMB3,LAMC2,LCA5,LDLR,LDLRAP1,LIFR,LIPA,LIPH,LOXHD1,LPL,LRPPRC,LYST,MAN2B1,MAT1A,MCCC1,MCCC2,MCOLN1,MECP2,MED17,MEFV,MESP2,MFSD8,MKS1,MLC1,MLYCD,MMAA,MMAB,MMACHC,MMADHC,MOCS1,MPI,MPL,MPV17,MRE11,MTHFR,MTM1,MTRR,MTTP,MUT(MMUT),MYO15A,MYO7A,NAGLU,NAGS,NBN,NDRG1,NDUFAF5,NDUFS4,NDUFS6,NEB,NEU1,NPC1,NPC2,NPHP1,NPHS1,NPHS2,NR2E3,NTRK1,OAT,OCRL,OPA3,OTC,PAH,PANK2,PC,PCCA,PCCB,PCDH15,PDHA1,PDHB,PEPD,PET100,PEX1,PEX10,PEX12,PEX2,PEX6,PEX7,PFKM,PHGDH,PIGN,PKHD1,PLA2G6,PNPO,POLG,POLH,POMGNT1,POR,PPT1,PREPL,PRPS1,PSAP,PTS,PUS1,PYGM,RAB23,RAG1,RAG2,RAPSN,RARS2,RDH12,RLBP1,RMRP(NME1),RNASEH2C,RPE65,RPGRIP1L,RS1,RTEL1,SACS,SAMD9,SAMHD1,SBDS,SEPSECS,SERPINA1,SGCA,SGCB,SGCD,SGCG,SGSH,SLC12A3,SLC12A6,SLC17A5,SLC19A2,SLC22A5,SLC25A13,SLC25A15,SLC25A20,SLC26A2,SLC26A3,SLC26A4,SLC35A3,SLC37A4,SLC39A4,SLC3A1,SLC45A2,SLC4A11,SLC6A8,SLC7A7,SLC7A9,SMARCAL1,SMN1,SMPD1,ST3GAL5,STAR,STRC,SUCLA2,SUMF1,SURF1,TAT,TCIRG1,TECPR2,TFR2,TGM1,TH,TMC1,TMEM216,TPO,TPP1,TREX1,TRIM32,TRMU,TSEN54,TSFM,TSHB,TSHR,TTC37,TTN,TTPA,TYMP,TYR,TYRP1,UGT1A1,UPB1,USH1C,USH2A,VPS13A,VPS13B,VPS45,VPS53,VRK1,VSX2,VWF,WAS,WISP3(CCN6),WNT10A,WRN,XPA,XPC,ZFYVE26,AKR1C4,AXL,BBS5,BBS7,CAPN10,DUSP6,EIF2B1,EIF2B3,F10,F12,F13A1,F13B,F2R,F5,F7,FGA,FGB,FGF17,FGFR2,GNAS,HOXA13,HS6ST1,INS,INSR,IRS1,IRS2,ITGA2,KLKB1,LEP,LEPR,LMNA,MTR,NOS1,PCSK1,PLAT,PLG,PRLR,PROC,PROCR,PROS1,RSPO1,SEMA3E,SERPINC1,SERPINE1,SERPINF1,SHBG,SOX10,SOX9,SPRY4,SRA1,THBD,TTC8,WWOX,ABCA3,AFF2,AHI1,ANO10,ARX,CC2D2A,CCDC88C,CLCN1,DYNC2H1,ELP1,FMO3,FMR1,FXN,G6PC1(G6PC),GALT,GRIP1,L1CAM,LRP2,MCPH1,MID1,MMUT,MVK,NAGA,OCA2,PLP1,PMM2,PRF1,RNASEH2B,RPGR,SCO2,SLC19A3,TF,TNXB,APC,MYH11,ACTA2,TMEM43,DSP,PKP2,DSG2,DSC2,BRCA2,SCN5A,RYR2,FLNC,MYBPC3,COL3A1,APOB,MYH7,TPM1,PRKAG2,TNNI3,MYL3,MYL2,ACTC1,RET,PALB2,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,WT1,FSHR,SRY,CYP11B1,NOBOX,GDF9,DLK1,DNMT1,FOXL2,SOHLH1,C3,FIGLA,BMP15,MCM8,MCM9,PSMC3IP,TRIM37,TG,IGSF10,MRPS22,NR5A1,MSH5,ERCC6,BMPR1B,GREM1,NOTCH2,STAG3,CAV1,NUP107,ATG7,ATG9A,ESR2,KHDRBS1,PGRMC1,SPIDR,POF1B,EIF2B2,EIF2B4,EIF2B5,HFM1,SYCE1,TGFBR3,POU5F1,CITED2,NANOS3,EIF4ENIF1,NOG,C14orh39,RAD51B,NPPC,FANCL,TP63,BUB1B,IL17RD,FLRT3,POLR3A,TUBB3,RAB3GAP2,SLC29A3,DCAF17,ALMS1,BBS12,MKKS,RAB3GAP1,PHF6,ARL6,FEZF1,PROK2,NDNF,KISS1R,GNRHR,KISS1,CCDC141,WDR11,TAC3,SOX2,TACR3,GNRH1,CADM1*

FEMALE POLYMORPHISM RSID

rs4148211, rs429358, rs7412, rs6165, rs1260326, rs1695, rs5911, rs5918, rs2293275, rs1801131, rs1801394, rs167479, rs1800961, rs6166, rs1801106, rs2341097, rs1805087, rs1801133, rs6578185, rs2278868, rs2276314, rs2653414, rs13394619, rs6065, rs1800595, rs5985, rs56381411, rs2276314

Recommendations

- Genetic counseling is recommended.

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.

Sequence data attributes

Total reads generated	15.04 Gb
Data ≥ Q30	94.51 %

Genetic test results are reported based on the recommendations of American College of Medical Genetics [1], as described below:

Classification	Interpretation
Pathogenic	A disease-causing variation in a gene which can explain the patients' symptoms has been detected. This usually means that a suspected disorder for which testing had been requested has been confirmed
Likely Pathogenic	A variant which is very likely to contribute to the development of disease however, the scientific evidence is currently insufficient to prove this conclusively. Additional evidence is expected to confirm this assertion of pathogenicity.
Variant of Uncertain Significance	A variant has been detected, but it is difficult to classify it as either pathogenic (disease causing) or benign (non- disease causing) based on current available scientific evidence. Further testing of the patient or family members as recommended by your clinician may be needed. It is probable that their significance can be assessed only with time, subject to availability of scientific evidence.

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

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This report has been reviewed and approved by:

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