THE UNIVERSITY OF CHICAGO | Genetic Services Laboratories

Next Generation Sequencing Panel for Premature Ovarian Failure

Clinical Features:

Premature Ovarian Failure (POF, also known as primary ovarian insufficiency), is the loss of predictable ovarian follicle function resulting in amenorrhea before the age of 40[1, 2]. Patients often have follicle-stimulating hormone (FSH) levels in the postmenopausal range[3]. Ovarian function may be intermittant, and spontaneous conceptions have been reported in 5-10% of patients with POF[1]. There are several etiologies of premature ovarian failure, including endocrinopathies, autoimmune disease, chromosomal abnormalities, and gene mutations[3]. Approximately 10% of cases of POF are familial[2]. It is estimated that 3-12% of women with premature ovarian failure carry a premutation in the *FMR1* gene [4].

Our Premature Ovarian Failure Panel includes sequencing and deletion/duplication analysis of the genes listed below, as well as repeat expansion testing of the *FMR1* gene.

Premature Ovarian Failure Panel			
BMP15	FSHR	MCM8	PSMC3IP
FIGLA	HFM1	NOBOX	
FOXL2	LMNA	NR5A1	

Gene	Clinical Features	Details
BMP15	Premature Ovarian Failure, Ovarian Dysgenesis	Nonconservative heterozygous mutations in *BMP15* have been identified in patients with POF. The majority of mutations are found in the proregion of the *BMP15* pre-protein and appear to reduce the production of mature *BMP15* peptide dimers[5].
FIGLA	Premature Ovarian Failure	Heterozygous mutations in *FIGLA* have been described in two patients with POF. Both mutations identified are deletions; one results in premature termination of the protein while the other removed a single amino acid altering binding *of FIGLA* to TCF3[6].
FOXL2	Blepharophimosis, Epicanthus Inversus and Ptosis syndrome (BPES), Premature ovarian failure	BPES is an autosomal dominant disorder characterized by eye abnormalities and facial features, and POF is observed in type I BPES[7]. A wide range of *FOXL2* mutations have been observed in patients with BPES type I as well as isolated POF[7].
FSHR	Ovarian Dysgenesis, Ovarian Hyperstimulation syndrome	Mutations in the *FSHR* gene have been found in patients with ovarian dysgenesis with an apparently recessive inheritance pattern[8, 9]. Ovarian hyperstimulation syndrome, characterized by hyper-reactive luteinized cells during pregnancy, has also been linked to *FSHR* mutations[10].
HFM1	Premature Ovarian Failure	Compound heterozygous mutations of *HFM1* have been identified in several patients with POF, including missense and splice site mutations[11].
LMNA	Malouf syndrome	Malouf syndrome is a cardiomyopathy syndrome with symptoms of dilated cardiomyopathy, mitral or tricuspid valve insufficiency, lipodystrophy and premature ovarian failure[12]. Malouf syndrome is inherited in an autosomal dominant manner and is associated with a wide spectrum of mutations in *LMNA.*
MCM8	Premature Ovarian Failure	A homozygous missense mutation in *MCM8* was identified in three sisters with POF[13]. Functional studies demonstrated decreased function of the mutant protein in chromosome break repair[13].
NOBOX	Premature Ovarian Failure	*NOBOX*-associated POF is an autosomal dominant form of POF that is characterized by reduced or absent follicles, delayed or absent puberty and amenorrhea[14, 15].
NR5A1	Premature Ovarian Failure, 46,XY Sex Reversal	*NR5A1* mutations have been observed in families with 46,XX POF and 46,XY disorder of sexual development in the same family and are associated with both phenotypes[16].
PSMC3IP	Ovarian Dysgenesis	A homozygous in-frame deletion in *PSMC3IP* was found to segregate in a large family with ovarian dysgenesis[17].

Premature Ovarian Failure Panel (Sequencing and deletion/duplication analysis plus repeat expansion analysis of FMR1)

Sample specifications:	3 to10 cc of blood in a purple top (EDTA) tube
Cost:	$2000
CPT codes:	81401, 81407
Turn-around time:	8 weeks

Test methods:

Comprehensive sequence coverage of the coding regions and splice junctions of all genes in this panel is performed. Targets of interest are enriched and prepared for sequencing using the Agilent SureSelect system. Sequencing is performed using Illumina technology and reads are aligned to the reference sequence. Variants are identified and evaluated using a custom collection of bioinformatic tools and comprehensively interpreted by our team of directors and genetic counselors. All novel and/or potentially pathogenic variants are confirmed by Sanger sequencing. The technical sensitivity of this test is estimated to be >99% for single nucleotide changes and insertions and deletions of less than 20 bp. Copy number variation (CNV) analysis of the coding region of the panel genes was performed by analysis of next generation sequencing datasets. We utilize CNV calling algorithms that compare the mean read depth in the sample against a reference dataset. This assay is designed to detect copy number variations involving one or more exons of the panel genes. All reported CNVs have been confirmed by other methodologies including Multiplex Ligation-dependent Probe Amplification (MLPA), array-CGH or quantitative Real Time PCR analysis. Repeat sizing is performed by standard flanking-PCR (F-PCR) and repeat primed PCR (RP-PCR) followed by capillary electrophoresis. F-PCR amplifies across the repeat region while RP-PCR amplifies within the repeat region. RP-PCR will detect large expansions that may not be detected by F-PCR and provides more accurate repeat sizing information. RP-PCR is performed using a fluorescently labeled primer specific to the target of interest, a “repeat primer” consisting of multiple repeats in tandem, and an anchor primer specific to a tail attached to the repeat primer. A ‘ladder’ of repeat size products is generated and sizing determined by counting the number of peaks of the ladder (Warner *et al.*, 1996. J Med Genet. 33(12):1022-1026). Expansions larger than 100 repeats for all the repeats tested can be detected but may not be sized by this test. For repeat sizes in the normal range the accuracy of the assay is +/- 1 repeat. For repeat sizes in the uncertain significance/reduced penetrance range and full mutation expansions that can be sized the accuracy of the assay is +/- 3 repeats.

Results:

Results, along with an interpretive report, will be faxed to the referring physician. Additional reports will be provided as requested. All abnormal results will be reported by telephone.

For more information about our testing options, please visit our website at dnatesting.uchicago.edu or contact us at 773-834-0555.

References:

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