Circadian and Complex Sleep Disorders Gene Sequencing Panel

Genes Tested:

ADCY3, ADK, ADORA2A, ADRB1, AK5, APP, ARNTL, ARNTL2, ATP2B3, BDNF, BHLHE40, BHLHE41, BLOC1S6, BTBD9, CACNA1A, CACNA1B, CACNA1G, CAMK2A, CAMK2B, CAMTA1, CDKL5, CHRM1, CHRM3, CIART, CLOCK, CNTNAP2, CREB1, CREBBP, CRH, CRY1, CRY2, CSNK1A1, CSNK1D, CSNK1E, CUL3, DBH, DBP, DISC1, EGR3, ELP3, ERC2, FAAH, FABP7, FBXL3, FMR1, FOS, FOSB, FOXP1, FTO, FUS, GRIA1, GRIA3, GRIN1, GRM1, GRM2, GRM3, HCRT, HCRTR2, HDC, HLF, HOMER1, HOMER2, HTR1A, HTR1B, HTR2A, HTR2C, HTR7, HTT, IFNAR1, IL1R1, IL6, JAML, KANSL1, KCNA2, KCNA3, KCNC1, KCNK9, KCNN3, KCTD5, KPNB1, LEP, MAP2K5, MCHR1, MEIS1, MTOR, NALCN, NCKAP5, NFKB1, NLGN2, NLGN3, NLRP3, NOS1, NPAS2, NPRL3, NPSR1, NR1D1, NR1D2, NTSR1, OPN4, OPRM1, PANX1, PAX8, PCDHA3, PDE4D, PER1, PER2, PER3, PPARGC1A, PPP3CA, PPP3R1, PRKAB2, PRKG1, PRL, PRNP, PROK2, PTPA, PTPRD, RAB3A, RCAN2, RGS16, RIMS1, RORA, RORB, RORC, SCN1A, SHANK3, SHMT1, SIK3, SLC18A2, SLC29A1, SLC6A2, SLC6A3, SLC6A4, TEF, TIMELESS, TNF, TNFRSF1A, TNRC6B, TOX3, TRANK1, UBB, UBE3A, VAMP2

Description:

The Circadian and Complex Sleep Disorders Gene Sequencing Panel utilizes whole exome sequencing (WES) technology to identify genetic variations in a predefined list of 143 genes that are known to be associated with circadian disorders and other sleep disorders in humans or have been shown to result in circadian dysfunction in animals. The gene list was developed through careful review of available evidence in the literature, and collaboration with scientists and physicians at Cincinnati Children's Circadian Medicine Clinic, which is the only clinic in the country dedicated to childhood circadian disorders. This gene sequencing panel is aimed to help pinpoint specific genes that cause circadian problems or put individuals at risk for general sleep disorders. Compared to WES, this targeted approach results in a shorter turnaround time and decreased cost. This test will be performed on the proband only and will not include the identification of ACMG recommended actionable incidental findings.

Indications:

- Sleep disorders in children including insomnia, restless sleep, excessive daytime sleepiness, frequent nighttime awakenings
- Circadian sleep disorders such as advanced, delayed, irregular, or non-24-hour sleep-wake rhythm disorders
- Complex disorders involving changes in sleep patterns and/or daytime symptoms that are not identifiable as a single sleep disorder

What is Reported?

Variants that will be discussed in detail in the report:

- Pathogenic, likely pathogenic variants and variants of uncertain clinical significance in Tier 1 genes*

Variants that will be listed in the report:

- Variants of uncertain clinical significance in Tier 2 and Tier 3 genes*
- Variants that predict an increased risk of diseases, but do not cause a disease by themselves (risk alleles).

*See Gene Categories on page 2 for gene tier information

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What is not reported:

- Variants in genes not included in the predefined gene list
- Variants classified as benign or likely benign*
- Variants in Tier 2 and Tier 3 genes with allele frequency >1% in genome aggregation database*

*See Gene Categories below for gene tier information

Gene Categories:

Based on the current evidence of human disease association, the genes on the Circadian and Complex Sleep Disorders Gene Sequencing Panel are grouped into three categories: Tier 1, Tier 2, and Tier 3. Tier 1 contains 27 genes known to be associated with human conditions affecting sleep. Tier 2 consists of 90 genes that show evidence in influencing circadian/sleep disorders in non-human mammalians. Tier 3 has 26 genes with evidence of influencing circadian rhythms in non-mammalian organisms, mainly in drosophila. The genes in Tier 2 and 3 may be upgraded to Tier 1 as additional knowledge emerges in human circadian medicine.

*(The genes in **pink** font in each of the 3 tables are clock and clock regulated genes.)*

Tier 1:

ADRB1	ARNTL	BHLHE41	CAMTA1	CIART
CREBBP	CRY1	CRY2	CSNK1D	FTO
GRIA3	GRM1	HCRT	KANSL1	NPSR1
OPRM1	PAX8	PCDHA3	PDE4D	PER1
PER2	PER3	PRNP	SLC18A2	TIMELESS
TRANK1	UBE3A			

Tier 2:

ADCY3	ADK	ADORA2A	AK5	APP
ATP2B3	BDNF	BLOC1S6	BTBD9	CACNA1A
CACNA1B	CACNA1G	CAMK2A	CAMK2B	CDKL5
CHRM1	CHRM3	CLOCK	CNTNAP2	CREB1
CRH	CSNK1E	DBH	DBP	DISC1
EGR3	ERC2	FAAH	FMR1	FOS
FOSB	FOXP1	FUS	GRIA1	GRM2
GRM3	HCRTR2	HDC	HOMER1	HTR1A

Tier 2 (continued):

HTR1B	HTR2A	HTR2C	HTR7	IFNAR1
IL1R1	IL6	JAML	KCNA2	KCNC1
KCNK9	KCNN3	LEP	MAP2K5	MCHR1
MEIS1	MTOR	NALCN	NCKAP5	NFKB1
NLGN2	NLGN3	NLRP3	NOS1	NPAS2
NPRL3	NTSR1	OPN4	PANX1	PRKG1
PRL	PROK2	PTPA	PTPRD	RAB3A
RGS16	RIMS1	SCN1A	SHANK3	SIK3
SLC29A1	SLC6A2	SLC6A3	SLC6A4	TNF
TNFRSF1A	TNRC6B	TOX3	UBB	VAMP2

Tier 3:

ARNTL2	BHLHE40	CSNK1A1	CUL3	ELP3
FABP7	FBXL3	GRIN1	HLF	HOMER2
HTT	KCNA3	KCTD5	KPNB1	NR1D1
NR1D2	PPARGC1A	PPP3CA	PPP3R1	PRKAB2
RCAN2	RORA	RORB	RORC	SHMT1
TEF				

Methodology:

The Circadian and Complex Sleep Disorders Gene Sequencing Panel uses the Human Comprehensive Exome kit to capture the exonic regions of genes from the genomic DNA extracted from the patient's specimen. Targeted regions are sequenced using an Illumina sequencing system with paired end reads. Sequence reads are aligned to the human reference genome (build UCSC hg19). Variants within exons and flanking sequences are identified and evaluated by an internally developed and validated bioinformatics analysis pipeline and Fabric Genomic Analysis platform. Data quality is assessed to confirm it has a minimum coverage of 20X for >95% of targets of interest.

Technical Limitations:

- Pathogenic variants may be present in a portion of the genes not covered by this test or in regions with suboptimal data due to homologous issue, polynucleotides, or nucleotide repeats, and therefore may not be identified. Thus, the absence of identified pathogenic variants does not exclude

the possibility of a genetic etiology for the patient's symptoms.

- Certain types of variants are not detected. Only single base pair changes or small insertions or deletions of DNA are detected. Large deletions, duplications, or rearrangements, mitochondrial genome mutations, repeat expansions, low level mosaicism and many epigenetic defects may not be detected by this test.

Regions of Homology:

These gene regions with homology may generate suboptimal data with potential false negative results.

GENE	CHROM	TRANSCRIPT	EXON	EXON_START	EXON_END	LENGTH	TIER
KCTD5	16	NM_018992	2	2745935	2746044	110 bps	2

Low Coverage (<20X) Regions*

GENE	CHROM	TRANSCRIPT	EXON	EXON_START	EXON_END	LENGTH	TIER
DISC1	1	NM_001164537	4	231881149	231881245	97 bps	2
DISC1	1	NM_001164539	10	231990484	231990771	288 bps	2
GRIA3	X	NM_000828	9	122536844	122536949	106 bps	1
SHANK3	22	NM_033517	11	51135984	51135989	6 bps	2

**These regions represent the low coverage (<20X) regions identified during our test validation. For specific patient cases, these regions may vary slightly.*

Turn-Around Time:

56 days (8 weeks)

Specimen:

The following specimens are accepted for this assay:

- 3 mls whole blood in a lavender top (EDTA) tube
- Saliva in an Oragene saliva kit. Please call 513-636-4474 for a free saliva collection kit.
- 10 mcg of high quality DNA extracted in a CLIA certified lab
- 25 mL amniotic fluid or two (2) T25 flasks grown to confluence

Label the tube with the patient's name, birth date, and date of collection.

CPT Codes:

- 81404, 81405, 81479 (x2)

Shipping Instructions:

Please enclose test requisition with sample. **All information must be completed before sample can be processed.**

Place samples in Styrofoam mailer and ship at room temperature by overnight Federal Express to arrive Monday through Saturday.

Ship to:

Genetics and Genomics Diagnostic Laboratory
3333 Burnet Avenue NRB 1042
Cincinnati, OH 45229
513-636-4474

References:

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Ruben, M. D., Hogenesch, J. B., & Smith, D. F. (2019). Sleep and Circadian Medicine: Time of Day in the Neurologic Clinic. Neurologic clinics, 37(3), 615–629.

Full list of gene specific references can be available upon request.