THE UNIVERSITY OF CHICAGO | Genetic Services Laboratories

Next Generation Sequencing Panel for Hyperparathyroidism

Clinical features:
Hyperparathyroidism is caused by overactivity of one or more of four parathyroid glands in the body. This increase in parathyroid activity leads to increased levels of parathyroid hormone (PTH) in the bloodstream, which results in abnormal calcium levels in the blood and organs. Primary hyperparathyroidism is caused by enlargement of one of the parathyroid glands, which results in overproduction of PTH and hypercalcemia. Individuals with primary hyperparathyroidism are typically treated with surgery. Patients who have hyperparathyroidism due to another underlying condition are said to have secondary hyperparathyroidism. These individuals initially have low levels of calcium in the body, and over time an increase in PTH is seen. Common presenting symptoms of patients with hyperparathyroidism are skeletal abnormalities/fragility and kidney stones. Hyperparathyroidism can also affect organs other than the skeleton and kidneys. Neuromusuclar, gastrointestinal, cardiovascular, and psychiatric abnormalities have all been noted in patients with primary hyperparathyroidism (1).

Our Hyperparathyroidism Panel includes sequence and deletion/duplication analysis of the 8 genes listed below.

Hyperparathyroidism Sequencing Panel			
AP2S1	CDC73	GNA11	PTH1R
CASR	CDKN1B	MEN1	RET

Molecular genetics:
Heterozygous mutations in *CASR, GNA11* and *AP2S1* are associated with familial hypocalciuric hypercalcemia (FHH) types 1,2 and 3, respectively. This condition is similar to primary hyperparathyroidism in presentation, but is not correctable by surgery. FHH type 1, caused by loss-of-function mutations in the *CASR* gene, is the most common form of this condition. Mutations in *GNA11* and *AP2S1* are less common, but mutations in *AP2S1* have been reported to more severely affect calcium homeostasis when compared to individuals with *CASR* mutations (2). Homozygous inactivating mutations in the *CASR* gene cause a severe form of neonatal hyperparathyroidism. This form of hyperparathyroidism typically manifests within the first 6 months of life with severe hypercalcemia, bone demineralization and failure to thrive. Parathyroidectomy can be considered for these patients, based on the severity of their symptoms. Dominant *CASR* mutations have been reported in a small number of patients with neonatal hyperparathyrodisim (3).

Multiple endocrine neoplasia (MEN) types 1, 2A, and 4 are caused by heterozygous mutations in *MEN1*, *RET*, and *CDKN1B*, respectively. The most common presenting feature in patients with MEN1 is primary hyperparathyroidism, which affects nearly 100% of affected individuals by age 50. Tumors of the parathyroid, pancreatic islets, duodenal endocrine cells, and the pituitary gland are common, with a 94% penetrance by age 50. Patients with MEN1 are at risk for a range of other tumors throughout the body, which are, with the exception of gastrinomas, non-metastasizing (4). Multiple endrocrine neoplasia type 4 includes patients with a MEN1-like phenotype caused by dominant mutations in the *CDKN1B* gene. Studies have indicated that approximately 3% of individuals with MEN1-associated tumors carry heterozygous *CDKN1B* mutations. (5).

MEN2A is caused by heterozygous mutations in the *RET* gene. Patients with MEN2A have a 100% of developing medullary thyroid carcinoma (MTC) by age 70; the presence of MTC is the defining characteristic of MEN2A. Pheochromocytomas are present in 50% of affected patients, and 15-30% develop primary hyperparathyroidism (HPT). HPT in patients with MEN2A is typically mild, and may range from a single adenoma to marked hyperplasia (6).

Heterozygous mutations in *CDC73* gene (also known as *HRPT2)* are associated with a range of parathyroid abnormalities including familial isolated hyperparathyroidism, hyperparathyroid-jaw tumor syndrome and sporadic parathyroid carcinoma. Studies suggest that up to 7% of individuals with familial isolated hyperparathyroidism have heterozygous pathogenic variants in *CDC73.* A distinct syndrome known as hyperparathyroid-jaw tumor syndrome (HPT-JT) is characterized by primary hyperparathyroidism, typically caused by a single parathyroid adenoma or parathyroid carcinoma. Ossified fibrous tumors of the jaw are present in 30-40% of patients with HPT-JT syndrome. Kidney lesions and uterine rumors are also more common in individuals with *CDC73*-relaed HPT-JT syndrome(7).

Heterozygous mutations in *PTH1R* are associated with Murk Jansen type metaphyseal chondrodysplasia. This condition is characterized by severe short stature with bowed limbs, clinodactyly, prominent upper face and small mandible. Patients with this condition have normal or undetectable levels of parathyroid hormone. However, laboratory findings are very similar to hyperparathyroidism, and include: hypercalcemia, hypophosphatemia and increased renal excretion of phosphate, cAMP and

hydroxyproline (8-10). Most cases of *PTH1R*-related Jansen metaphyseal dysplasia are de novo; however, familial cases of the condition have been reported (11).

Test methods:
Comprehensive sequence coverage of the coding regions and splice junctions of all genes in this panel is performed. Targets of interests 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 pathogenic and likely 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.

Our CNV detection algorithm was developed and its performance determined for the sole purpose of identifying deletions and duplications within the coding region of the gene(s) tested. Partial exonic copy number changes and rearrangements of less than 400 bp may not be detected by this methodology. Regions of high homology and repetitive regions may not be analyzed. This methodology will not detect low level mosaicism, balanced translocations, inversions, or point mutations that may be responsible for the clinical phenotype. The sensitivity of our deletion/duplication assay may be reduced when DNA extracted by an outside laboratory is provided.

Hyperparathyroidism Panel (8 genes)

Sample specifications:	3 to10 cc of blood in a purple top (EDTA) tube
Cost:	$2500
CPT codes:	81406
	81407
Turn-around time:	4 weeks

Note: We cannot bill insurance for the above test.

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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