THE UNIVERSITY OF CHICAGO | Genetic Services Laboratories

Next Generation Sequencing Panel for Hypoparathyroidism

Clinical features:

Hypoparathyroidism is a rare condition in which the body secretes abnormally low levels of parathyroid hormone (PTH). PTH is responsible for regulation and maintenance of calcium and phosphorus in the body. The predominant clinical features of hypoparathyroidism are related to hypocalcemia. Acute episodes of hypocalcemia can lead to neuromuscular irritability, tingling and numbness of the mouth and extremities, muscle spasms and seizures. Individuals with chronic hypocalcemia may be asymptomatic. Other manifestations of hypoparathyroidism and hypocalcemia are premature cataracts, calcifications of the basal ganglia, impaired cardiac function, mental retardation and/or personality disorders (1). The most common cause of hypoparathyroidism is surgical resection or autoimmune destruction of the parathyroid. Iron overload of the parathyroid glands in patients with thalassaemia is another common cause of decreased parathyroid function. In rare cases, hypoparathyroidism is caused by an underlying genetic disorder (2). Identification of the etiology of hypoparathyroidism can aid in guiding clinical management of affected patients.

Our Hypoparathyroidism Sequencing Panel includes all 17 genes listed below.

Hypoparathyroidism Sequencing Panel			
AIRE	GATA3	HADHB	TBCE
CASR	GCM2	PDE4D	TBX1
CHD7	GNA11	PRKAR1A	
CYP24A1	GNAS	PTH	
FAM111A	HADHA	STX16	

Genes and Associated Disorder	Inheritance	Clinical Features/Molecular Pathology
AIRE [OMIM#607358] Autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APCED) [OMIM#240300]	AD/AR	Mutations in *AIRE* result in autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED). The most common features of APECED are chronic mucocutaneous candidiasis (CMC), hypoparathyroidism, and adrenal insufficiency. While most cases of APECED are caused by biallelic mutations in the *AIRE* gene, autosomal dominant inheritance has been reported (3)
CASR [OMIM#601199] Hypocalcemia with or without Bartter syndrome [OMIM#601198]	AD	CASR is a plasma membrane receptor expressed in the parathyroid hormone-producing cells of the parathyroid gland, and the cells lining the kidney tubule. It is essential in regulation of mineral ion homeostasis. Heterozygous mutations in *CASR* can cause an autosomal dominant form of hypocalcemia with or without Bartter syndrome (4).
CHD7 [OMIM#608892] CHARGE syndrome [OMIM#214800]	AD	Heterozygous mutations in *CHD7* are associated with CHARGE syndrome, a condition characterized by a pattern of congenital anomalies including choanal atresia, malformations of the heart, inner ear, and retina. There are several reports of patients with CHARGE syndrome who are also affected with DiGeorge sequence, including hypoparathyroidism (5, 6)
CYP24A1 [OMIM#126065] Infantile hypercalcemia-1 [OMIM#143880]	AR	The CYP24A1 enzyme is responsible for the inactivation of vitamin D derivative 1,25-dihydroxyvitamin D_{3}, and controlled by levels of 1,25-dihydroxyvitamin D, serum calcium, and parathyroid hormone. Biallelic mutations in *CYP24A1* are associated with idiopathic infantile onset hypercalcemia, which is characterized by severe hypercalcemia, failure to thrive, vomiting, dehydration and nephrocalcinosis. Laboratory evaluation of a cohort of infants presenting with these symptoms showed suppression of parathyroid hormone levels (7).

FAM111A [OMIM#615292] Kenny-Caffey syndrome type 2 [OMIM#127000] Gracile bone dysplasia [OMIM#602361	AD	Kenny-Caffey syndrome and gracile bone dysplasia are allelic autosomal dominant disorders causing abnormalities of skeletal development, hypoparathyroidism and hypocalcemia. Gracile bone dysplasia is typically perinatal lethal, and is characterized by gracile bones with thin diaphysis, premature closure of the basal cranial sutures, and microphthalmia. Kenny-Caffey syndrome is less severe, and is characterized by delayed closure of the fontanels, abnormal dentition, eye abnormalities and transient hypocalcemia. In a study by Unger, *et al*, 5 patients with Kenny-Caffey syndrome and 5 patients with gracile bone dysplasia were identified as heterozygous for 6 mutations in the *FAM111A*. For all families where parents were available, these mutations were confirmed to be *de* novo (8)
GATA3 [OMIM#131320] Hypoparathyroidism, sensorineural deafness, and renal dysplasia [OMIM#146255]	AD	Haploinsufficiency of the *GATA3* gene leads to hypoparathyroisim, sensorineural deafness and renal dysplasia (also known as HDR syndrome). In a study by Ali *et al,* 13 different mutations in *GATA3* were identified in 13 out of 21 probands with HDR syndrome. No mutations were identified in patients with isolated hypoparathyroidism, indicating that *GATA3* mutations are more likely to result in two or more phenotypic features of HDR syndrome (9)
GCM2 [OMIM#603716] *PTH* [OMIM#168450] Familial isolated hypoparathyroidism [OMIM#146200]	AD/AR	Isolated hypoparathyroidism can be caused by mutations in the parathyroid hormone gene (*PTH*) or in *GCM2*. While most cases of familial isolated hypoparathyroidism have been autosomal dominant in inheritance, there have been reports of autosomal recessive inheritance (10, 11)
GNA11 [OMIM#139313] Autosomal dominant hypocalcemia type 2 [OMIM#615361] Hypocalciuric hypercalcemia type II [OMIM#145981]	AD	Heterozygous mutations in *GNA11* have been identified in 5 different families with autosomal dominant hypocalcemia(12-14). In families where multiple generations were affected, these mutations segregated with disease. Nesbit, *et al* reported one in-frame deletion and one missense *GNA11* mutation in two unrelated families with hypocalciuric hypercalcemia. These patients were known to be negative for mutations in *CASR* and *AP2S1* (14).
GNAS [OMIM#139320] *STX16* [OMIM#603666] Pseudohypoparathyroidism, types IA [OMIM#103580], IB [OMIM#603233], IC [OMIM#612462] Pseudopseudohypoparathyroidism [OMIM#612463]	AD	Pseudohypoparathyroidism (PHP) is a term that defines of group of disorders which all feature parathyroid hormone resistance. Individuals with PHP type IA, caused by maternal loss-of function mutations in *GNAS,* are also resistant to thyroid stimulating hormone (TSH) and gonadotropins. The *GNAS* gene displays tissue-specific differential expression depending on the parent of origin. Only the maternal copy of *GNAS* is expressed in renal tubular cells. Therefore, inactivation of the maternal allele results in no expression in these cell lines, resulting in disease. This type of PHP is also associated with Albright hereditary osteodystrophy, which is defined by skeletal abnormalities such as short stature, subcutaneous ossifications and brachydactyly. PHP type IB is also caused by maternal mutations in the differentially methylated region of *GNAS*, and results in a PHP phenotype without the skeletal abnormalities seen in type 1A. PHP1B can also be caused by deletions in the *STX16* gene, a control element of methylation of *GNAS*. PHP type IC is similar to type IA in cause and phenotype, with the exception of retained Gs activity in erythrocytes. Pseudopseudohypoparathyroidism (PPHP) is caused by loss-of function mutations on the paternal allele of the *GNAS* gene. Individuals with PPHP do not show hormone resistance, but do have clinical features consistent with a diagnosis of Albright hereditary osteodystrophy, as seen in PHP type IA(15).
HADHA [OMIM#600890] *HADHB* [OMIM#143450] Mitochondrial trifunctional protein deficiency with myopathy and neuropathy [OMIM#609015]	AR	Mitochondrial trifunctional protein deficiency, caused by biallelic mutations in *HADHA* and *HADHB*, is characterized by decreased activity of long-chain-3-hodrosyacyl-CoA dehydrogenase (LCHAD), long-chain enoyl-CoA hydratase, and long-chain thiolase. These three enzymes are involved in mitochondrial beta-oxidation of fatty acids. Clinical presentation can range from sudden unexplained infant death (SIDS), infantile onset hepatic Reye-like syndrome, and late-adolescent

		onset skeletal myopathy. In vitro studies have indicated a genotype-phenotype correlation, with mutations resulting in no residual protein activity causing a more severe phenotype than those associated with residual activity (16).
PRKAR1A [OMIM#188830] *PDE4D* [OMIM#600129] Acrodysostosis types 1 and 2 [OMIM#s 101800 and 614613]	AD	Heterozygous mutations in *PRKAR1A* are associated with acrodysostosis-1, with or without hormone resistance. This form of skeletal dysplasia is characterized by short stature, severe brachydactyly, facial dysostosis and nasal hypoplasia. Hormone resistance, including PTH, is reported in a subset of affected individuals. Acrodysostosis type 2 is caused by heterozygous mutations in *PDE4D*, and is similar in phenotype to acrodysostosis type 1. Many patients with type 2 acrodysostosis have intellectual disability and, similar to type 1, some patients are affected with hormone resistance (17).
TBCE [OMIM#604934] Hypoparathyroidism-retardation-dysmorphism syndrome [OMIM#241410] Kenny-Caffey syndrome, type 1 [OMIM#244460]	AR	Bialllic mutations in *TBCE* can result in hypoparathyroidism-retardation-dysmorphism syndrome (HRDS). This condition is characterized by congenital hypoparathyroidism, mental retardation, facial dysmorphism and growth failure. This condition typically affects individuals from Middle Eastern populations, and is often caused by a known founder mutation in the *TBCE* gene. Kenny-Caffey syndrome is similar in phenotype to HRDS, but also includes the presence of osteosclerosis and recurrent infections (18).
TBX1 [OMIM#602054] DiGeorge syndrome [OMIM#188400]	AD	*TBX1* is considered to be the key gene responsible for many of the features of 22q11.2 deletion syndrome, or DiGeorge syndrome. While this condition is most often caused by a recurrent microdeletion at the 22q11.2 locus, mutations in *TBX1* have been identified in patients with the characteristic features of DiGeorge syndrome and no identifiable deletion. DiGeorge syndrome is associated with heart defects, cleft palate, distinctive facial features, hearing loss, and hypocalcemia caused by parathyroid and thymic hypoplasia (19).

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. Deletion/duplication analysis of the panel genes is performed by oligonucleotide array-CGH. Partial exonic copy number changes and rearrangements of less than 400 bp may not be detected by array-CGH. Array-CGH will not detect low-level mosaicism, balanced translocations, inversions, or point mutations that may be responsible for the clinical phenotype. The sensitivity of this assay may be reduced when DNA is extracted by an outside laboratory.

Hypoparathyroidism Sequencing Panel (sequence analysis of 17 genes)

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

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.

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