



Next Generation Sequencing Panel for Congenital Hypothyroidism

Clinical Features:

Congenital hypothyroidism is a condition characterized by inadequate availability of active thyroid hormone to target tissues in the newborn period. It can be sporadic or inherited. In 80 to 85% of cases, congenital hypothyroidism is the consequence of thyroid dysgenesis due to absent (athyreosis), abnormally located (ectopic), or small (hypoplastic) thyroid gland (1). Congenital hypothyroidism may also be due to dyshormonogenesis, caused by a complete or partial defect in thyroid hormone synthesis (2). Rare causes of congenital hypothyroidism include defects in the effect of thyroid hormone on target tissues due to abnormalities in cell membrane transport, metabolism or action, and control of thyroid gland activity due to defects at the level of the hypothalamus or pituitary (central hypothyroidism) and more commonly the thyrotropin receptor. Congenital hypothyroidism may be a feature of a syndromic genetic condition; examples include Kabuki syndrome, Johanson-Blizzard syndrome, and Pendred syndrome. Congenital hypothyroidism may be transient or permanent. A genetic diagnosis can aid in determining whether lifelong thyroid hormone replacement is necessary (2), as well as provide risk information and the possibility of genetic counseling to families.

Our Congenital Hypothyroidism Sequencing Panel includes the 22 genes listed below.

Congenital Hypothyroidism Panel Genes					
DUOX2	IGSF1	NKX2-1	SLC16A2	THRA	TSHR
DUOXA2	IYD	PAX8	SLC26A4	THRB	UBR1
FOXE1	KDM6A	POU1F1	SLC5A5	TPO	
GLIS3	KMT2D	PROP1	TG	TSHB	

Condition	Genes	Clinical and Molecular Findings
Thyroid dysgenesis	<i>FOXE1 (TTF2)</i> [OMIM#602617] <i>GLIS3</i> [OMIM#610192] <i>NKX2-1 (TTF1)</i> [OMIM#600635] <i>PAX8</i> [OMIM#167415] <i>TSHR</i> [OMIM#603372]	Abnormalities of thyroid gland development during embryogenesis can lead to absent, hypoplastic or ectopic thyroid glands, resulting in hypothyroidism. In the majority of cases, the underlying cause of thyroid dysgenesis is unknown. However, a subset of cases have an identifiable genetic etiology (3). Single gene causes of thyroid dysgenesis may be syndromic or non-syndromic. Syndromic disorders of thyroid dysgenesis are caused by mutations in <i>FOXE1</i> , <i>GLIS3</i> , <i>NKX2-1</i> (for details see below). Non-syndromic thyroid dysgenesis may be caused by heterozygous mutations in <i>PAX8</i> (4). Biallelic inactivating mutations in the <i>TSHR</i> gene lead to congenital nongoiterous hypothyroidism due to resistance to thyroid stimulating hormone (4). These individuals may also have hypoplasia of the thyroid gland (5). Heterozygous mutations in <i>TSHR</i> may lead to a mild phenotype including hyperthyrotropinemia with a normal or small thyroid gland (3). Gain-of function mutations in <i>TSHR</i> lead to neonatal hyperthyroidism (6).
Thyroid dyshormonogenesis NIS (iodide trapping defect) TPO (iodide organification defect)	<i>DUOX2 (THOX2)</i> [OMIM#606759] <i>DUOXA2</i> [OMIM#612772] <i>SLC5A5 (NIS)</i> [OMIM#601843] <i>TG</i> [OMIM#188450] <i>TPO</i> [OMIM#606765] <i>IYD (DEHAL1)</i> [OMIM#612025]	Patients with thyroid dyshormonogenesis exhibit absent or reduced synthesis of thyroid hormone (2). They could be born with an enlarged thyroid gland or may develop goiter later in life. Infants with thyroid dyshormonogenesis are typically detected by newborn screening. However, newborns with biallelic defects in the <i>IYD</i> gene do not exhibit an iodide deficiency at birth and therefore are not identified by newborn screening (7). Several genes are implicated in thyroid dyshormonogenesis, the majority of which have autosomal recessive inheritance. One exception is <i>DUOX2</i> ; heterozygous mutations in <i>DUOX2</i> can lead to congenital hypothyroidism that is transient, whereas biallelic mutations in <i>DUOX2</i> typically lead to permanent congenital hypothyroidism (7). Identifying the underlying defect responsible for thyroid dyshormonogenesis can influence the decision to treat as well as the approach to treatment. For example, individuals with <i>SLC5A5</i> -related hypothyroidism may show improved thyroid function with iodide

		supplementation alone (7). Patients with transient hypothyroidism due to a heterozygous mutation in <i>DUOX2</i> may not require lifelong thyroid supplementation, but these individuals should be monitored for recurrent hypothyroidism (7).
Congenital hypothyroidism secondary to combined pituitary hormone deficiency	<i>POU1F1</i> [OMIM#173110] <i>PROP1</i> [OMIM#601538]	Combined pituitary hormone deficiency (CPHD) is a condition associated with deficiency of one or more of the hormones produced by the anterior pituitary gland, including growth hormone (GH), thyroid-stimulating hormone (TSH), luteinizing hormone (LH), follicle-stimulating hormone (FSH), prolactin (PRL), and adrenocorticotropic hormone (ACTH). Clinical findings of CPHD can include growth failure, failure to thrive, delayed or absent puberty, and infertility. Hypothyroidism may be congenital or may occur in late infancy or childhood (8, 9). <i>PROP1</i> -related CPHD is inherited in an autosomal recessive manner. <i>POU1F1</i> -related CPHD can be inherited in either an autosomal recessive or an autosomal dominant manner.
Thyroid hormone receptor defects: Resistance to thyroid hormone beta (RTH β , Refetoff syndrome) Resistance to thyroid hormone alpha (RTH α)	<i>THRB</i> [OMIM#190160] <i>THRA</i> [OMIM#190120]	Congenital hypothyroidism due to resistance to thyroid hormone (RTH) is caused by defects in the thyroid nuclear receptors. The latter are transcription factors that regulate thyroid hormone-mediated gene expression and are activated by thyroid hormone binding (10). Thyroid hormone receptors are encoded for by the thyroid hormone receptor beta (<i>THRB</i>) and thyroid hormone receptor alpha (<i>THRA</i>) genes. The majority of cases of resistance to thyroid hormone are due to mutations in the <i>THRB</i> gene (resistance to thyroid hormone beta, RTH β). Several consanguineous families have been reported with homozygous mutations in <i>THRB</i> (11-14); these patients exhibited a variety of extrathyroidal features including intellectual disability, tachycardia, congenital hearing loss, and epiphyseal dysgenesis. The remaining reported cases of RTH β are autosomal dominant and mutations have a dominant-negative effect (12). These patients exhibit generalized resistance to thyroid hormone and variable learning disabilities, behavioral abnormalities or developmental delays. Heterozygous mutations in <i>THRA</i> have been reported in a subset of patients with resistance to thyroid hormone with a distinct phenotype characterized by developmental delay, constipation, skeletal dysplasia, macrocephaly and anemia (resistance to thyroid hormone alpha, RTH α (15, 16). <i>De novo</i> mutations occur in both forms of thyroid hormone resistance, particularly in CpG hot spots.
Brain-lung-thyroid syndrome	<i>NKX2-1 (TTF1)</i> [OMIM#600635]	Heterozygous pathogenic mutations in <i>NKX2-1</i> are associated with brain-lung-thyroid syndrome, also known as choreoathetosis, congenital hypothyroidism, and neonatal respiratory distress (17). This condition is characterized by thyroid dysfunction due to thyroid dysgenesis, in addition to neurological features and pulmonary dysfunction. Neurological features include chorea, tremor, and dysarthria. Heterozygous mutations in <i>NKX2-1</i> have also been reported in individuals with only benign hereditary chorea (18). Most individuals with <i>NKX2-1</i> -related disorders have an affected parent, though <i>de novo</i> cases have been reported (17).
Pendred syndrome	<i>SLC26A4</i> [OMIM#605646]	Pendred syndrome is a syndromic form of congenital hearing loss characterized by severe to profound hearing loss that is accompanied by abnormalities of the inner ear including abnormalities of the temporal bones, cochlea, and enlargement of the vestibular aqueduct. The majority of patients develop goiter, with 40% developing goiter in late childhood or early in puberty (19). Pendred syndrome is an autosomal recessive condition caused by homozygous or compound heterozygous mutations in the <i>SLC26A4</i> gene. Mutations in <i>SLC26A4</i> are also associated with non-syndromic hearing loss (DFNB4) (19).
Johanson-Blizzard syndrome	<i>UBR1</i> [OMIM#605981]	Johanson-Blizzard syndrome is an autosomal recessive multisystem disorder characterized by exocrine pancreatic insufficiency, dental anomalies and aplasia or hypoplasia of the nasal alae. Other variable features include aplasia cutis congenita, growth retardation, and sensorineural hearing loss (20). Sukalo <i>et al.</i> , 2014 identified hypothyroidism in 39% of Johanson-Blizzard patients from 50 unrelated families (20).

Neonatal diabetes and hypothyroidism	<i>GLIS3</i> [OMIM#610192]	Neonatal diabetes mellitus with congenital hypothyroidism (NDH) syndrome is characterized by neonatal diabetes mellitus, severe congenital hypothyroidism, hepatic fibrosis, polycystic kidneys and congenital glaucoma (21). Facial dysmorphism, intrauterine growth restriction and mild intellectual disability have also been reported (22). NDH syndrome is an autosomal recessive condition caused by homozygous or compound heterozygous mutations in the <i>GLIS3</i> gene. To date, frameshift mutations and gross deletions have been described (22). <i>GLIS3</i> belongs to the GLIS subfamily of Kruppel-like zinc finger proteins and functions as an activator and repressor of transcription.
Central hypothyroidism	<i>IGSF1</i> [OMIM#300137] <i>TSHB</i> [OMIM#188540]	Central hypothyroidism is a reduction of circulating thyroid hormone due to inadequate stimulation of a normal thyroid gland by TSH. Hemizygous mutations in <i>IGSF1</i> are associated with central hypothyroidism and testicular enlargement (23). A subset of female carriers exhibit central hypothyroidism (24). Biallelic mutations in <i>TSHB</i> , the beta subunit of thyroid-stimulating hormone, may also lead to central hypothyroidism (25).
Kabuki syndrome	<i>KDM6A</i> [OMIM#300128] <i>KMT2D</i> [OMIM#602113]	Patients with Kabuki syndrome have characteristic facial features, short stature, congenital heart defects, skeletal anomalies, immunological abnormalities, and mild to moderate mental retardation (26). Kawame, <i>et al.</i> , 1999, reported congenital hypothyroidism in 3 of 18 patients with a clinical diagnosis of Kabuki syndrome (27). Kabuki syndrome is caused by mutations in <i>KMT2D</i> or <i>KDM6A</i> . <i>KMT2D</i> -related Kabuki syndrome is an autosomal dominant condition mainly due to <i>de novo</i> mutations. <i>KDM6A</i> -related Kabuki syndrome is an X-linked disorder. To date, all reported cases of <i>KDM6A</i> -related Kabuki syndrome have been <i>de novo</i> .
Thyroid hormone cell membrane transport defect (Allan-Herndon-Dudley syndrome)	<i>SLC16A2 (MCT8)</i> [OMIM#300095]	Allan-Herndon-Dudley syndrome is caused by mutations of the <i>SLC16A2</i> gene. It encodes the monocarboxylate transporter 8 (MCT8), a specific thyroid hormone cell transporter (THCT). Allan-Herndon-Dudley syndrome is an X-linked condition that almost exclusively affects males. It is characterized by severe psychomotor delays and abnormal thyroid function. Affected males have severe developmental delay, gait disturbance, dystonia, and poor head control, in addition to high serum T3 and low reverse T3 levels. The majority of affected individuals cannot walk or talk. Although death of affected males in the early teens is not uncommon, males with mild T3 transport defect have survived to old age. Hypotonia is typical in early infancy, spasticity develops in late childhood with dystonic/ataxic movements and garbled or no speech. Heterozygous carrier females have only mild thyroid hormone abnormalities but no neuropsychiatric defects (28).
Hypothyroidism, thyroidal or athyroidal, with spiky hair and cleft palate (Bamforth-Lazarus syndrome)	<i>FOXE1 (TTF2)</i> [OMIM#602617]	Bamforth-Lazarus syndrome is characterized by hypothyroidism due to severe underdevelopment or complete absence of the thyroid gland, in association with choanal atresia, bifid epiglottis, cleft palate, and spiky or curly hair. The majority of reported patients have thyroid atrophy (3). Polyhydramnios is a common prenatal finding. Homozygous missense mutations in <i>FOXE1</i> have been identified in several consanguineous families with Bamforth-Lazarus syndrome (29-31).

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. Sequencing may not detect low level mosaicism.

Congenital Hypothyroidism Sequencing Panel (22 genes sequencing)

Sample specifications:	3 to 10 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.

References:

1. Macchia PE, Lapi P, Krude H et al. PAX8 mutations associated with congenital hypothyroidism caused by thyroid dysgenesis. *Nat Genet* 1998; 19: 83-86.
2. Grasberger H, Refetoff S. Genetic causes of congenital hypothyroidism due to dysmorphogenesis. *Curr Opin Pediatr* 2011; 23: 421-428.
3. Pohlenz J, Van Vliet G, Deladoey J. Developmental Abnormalities of the Thyroid. In: Weiss R, Refetoff S, eds. *Genetic Diagnosis of Endocrine Disorders*: Elsevier Inc., 2016: 127-136.
4. Szinnai G. Clinical genetics of congenital hypothyroidism. *Endocr Dev* 2014; 26: 60-78.
5. De Felice M, Di Lauro R. Thyroid development and its disorders: genetics and molecular mechanisms. *Endocr Rev* 2004; 25: 722-746.
6. Kopp P, van Sande J, Parma J et al. Brief report: congenital hyperthyroidism caused by a mutation in the thyrotropin-receptor gene. *N Engl J Med* 1995; 332: 150-154.
7. Grasberger H, Refetoff S. Congenital Defects of Thyroid Hormone Synthesis. In: Weiss R, Refetoff S, eds. *Genetic Diagnosis of Endocrine Disorders*: Elsevier, 2016: 117-125.
8. Hendriks-Stegeman BI, Augustijn KD, Bakker B et al. Combined pituitary hormone deficiency caused by compound heterozygosity for two novel mutations in the POU domain of the Pit1/POU1F1 gene. *J Clin Endocrinol Metab* 2001; 86: 1545-1550.
9. de Zegher F, Pernasetti F, Vanhole C et al. The prenatal role of thyroid hormone evidenced by fetomaternal Pit-1 deficiency. *J Clin Endocrinol Metab* 1995; 80: 3127-3130.
10. Nagaya T, Nomura Y, Fujieda M et al. Heterodimerization preferences of thyroid hormone receptor alpha isoforms. *Biochem Biophys Res Commun* 1996; 226: 426-430.
11. Refetoff S, DeWind LT, DeGroot LJ. Familial syndrome combining deaf-mutism, stunted epiphyses, goiter and abnormally high PBI: possible target organ refractoriness to thyroid hormone. *J Clin Endocrinol Metab* 1967; 27: 279-294.
12. Takeda K, Balzano S, Sakurai A et al. Screening of nineteen unrelated families with generalized resistance to thyroid hormone for known point mutations in the thyroid hormone receptor beta gene and the detection of a new mutation. *J Clin Invest* 1991; 87: 496-502.
13. Ferrara AM, Onigata K, Ercan O et al. Homozygous thyroid hormone receptor β -gene mutations in resistance to thyroid hormone: three new cases and review of the literature. *J Clin Endocrinol Metab* 2012; 97: 1328-1336.
14. Usala SJ, Menke JB, Watson TL et al. A homozygous deletion in the c-erbA beta thyroid hormone receptor gene in a patient with generalized thyroid hormone resistance: isolation and characterization of the mutant receptor. *Mol Endocrinol* 1991; 5: 327-335.
15. van Mullem A, van Heerebeek R, Chrysis D et al. Clinical phenotype and mutant TR α 1. *N Engl J Med* 2012; 366: 1451-1453.
16. Tyłki-Szymańska A, Acuna-Hidalgo R, Krajewska-Walasek M et al. Thyroid hormone resistance syndrome due to mutations in the thyroid hormone receptor α gene (THRA). *J Med Genet* 2015; 52: 312-316.
17. Patel N, Jankovic J. NKX2-1-Related Disorders. In: Pagon R, MP A, HH A et al., eds. *GeneReviews* [Internet]. Seattle, WA: University of Washington, 2014 [last updated 2016].
18. Breedveld GJ, van Dongen JW, Danesino C et al. Mutations in TITF-1 are associated with benign hereditary chorea. *Hum Mol Genet* 2002; 11: 971-979.
19. Alasti Fa, Van Camp G, Smith RJ. Pendred syndrome. In: Pagon R, Adam M, Ardinger H, eds. *GeneReviews* [internet]. Seattle, WA: University of Washington, Last updated 2014.
20. Sukalo M, Fiedler A, Guzmán C et al. Mutations in the human UBR1 gene and the associated phenotypic spectrum. *Hum Mutat* 2014; 35: 521-531.
21. Taha D, Barbar M, Kanaan H et al. Neonatal diabetes mellitus, congenital hypothyroidism, hepatic fibrosis, polycystic kidneys, and congenital glaucoma: a new autosomal recessive syndrome? *Am J Med Genet A* 2003; 122A: 269-273.
22. Senée V, Chelala C, Duchatelet S et al. Mutations in GLIS3 are responsible for a rare syndrome with neonatal diabetes mellitus and congenital hypothyroidism. *Nat Genet* 2006; 38: 682-687.
23. Sun Y, Bak B, Schoenmakers N et al. Loss-of-function mutations in IGSF1 cause an X-linked syndrome of central hypothyroidism and testicular enlargement. *Nat Genet* 2012; 44: 1375-1381.
24. Joustra SD, Schoenmakers N, Persani L et al. The IGSF1 deficiency syndrome: characteristics of male and female patients. *J Clin Endocrinol Metab* 2013; 98: 4942-4952.
25. Brumm H, Pfeufer A, Biebermann H et al. Congenital central hypothyroidism due to homozygous thyrotropin beta 313 Delta T mutation is caused by a Founder effect. *J Clin Endocrinol Metab* 2002; 87: 4811-4816.
26. Matsumoto N, Niikawa N. Kabuki make-up syndrome: a review. *Am J Med Genet C Semin Med Genet* 2003; 117C: 57-65.
27. Kawame H, Hannibal MC, Hudgins L et al. Phenotypic spectrum and management issues in Kabuki syndrome. *J Pediatr* 1999; 134: 480-485.
28. Weiss R, Dumitrescu A, Refetoff S. Syndromes of Impaired Sensitivity to Thyroid Hormone. In: Weiss R, Refetoff S, eds. *Genetic Diagnosis of Endocrine Disorders*: Elsevier Inc., 2016: 137-151.
29. Clifton-Bligh RJ, Wentworth JM, Heinz P et al. Mutation of the gene encoding human TTF-2 associated with thyroid agenesis, cleft palate and choanal atresia. *Nat Genet* 1998; 19: 399-401.
30. Castanet M, Park SM, Smith A et al. A novel loss-of-function mutation in TTF-2 is associated with congenital hypothyroidism, thyroid agenesis and cleft palate. *Hum Mol Genet* 2002; 11: 2051-2059.
31. Baris I, Arisoy AE, Smith A et al. A novel missense mutation in human TTF-2 (FKHL15) gene associated with congenital hypothyroidism but not athyreosis. *J Clin Endocrinol Metab* 2006; 91: 4183-4187.

Committed to CUSTOMIZED DIAGNOSTICS, TRANSLATIONAL RESEARCH & YOUR PATIENTS' NEEDS