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Clinical Relevance of Molecular Diagnosis in Patients with Congenital Hypothyroidism

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Abstract

Congenital hypothyroidism, defined as the functional deficiency of thyroid hormones present at birth, occurs in approximately 1: 2,000 to 4,000 newborns. Thyroid hormones play an essential role in the maturation of the central nervous system. Congenital hypothyroidism results in severe neurodevelopmental impairment if untreated and, therefore constitutes the most common preventable endocrine cause of irreversible mental retardation. As clinical diagnosis of hypothyroidism in the newborn period is almost always overlooked, newborn screening programs seeking to identify elevated thyrotropin levels at birth are available to detect primary congenital hypothyroidism mainly. Significantly, early onset on levothyroxine replacement therapy virtually abolishes severe intellectual development.

Congenital hypothyroidism is caused by genetic defects occurring at three different levels, including the hypothalamic-pituitary axis, the thyroid gland, and the peripheral tissues. Up to date, 30 monogenic forms of congenital hypothyroidism have been reported in individuals with thyroid dysgenesis, thyroid dyshormonogenesis, central and peripheral hypothyroidism, highlighting the genetic heterogeneity of the disease.

This mini-review summarizes the latest advances in the genetic basis of monogenic forms of congenital hypothyroidism and novel strategies to uncover the molecular etiology of the disease. Moreover, the article provides the current knowledge and future perspectives on the clinical relevance of the molecular diagnosis in patients with congenital hypothyroidism.

Keywords: Congenital hypothyroidism; Thyroid development; Thyroid hormone biosynthesis; Thyroid hormone action; Cell-membrane transport; Metabolism; Sanger sequencing; Targeted next-generation sequencing; Whole-exome sequencing

Congenital Hypothyroidism

Congenital hypothyroidism, defined as the functional deficiency of thyroid hormones present at birth, is the most frequent endocrine disorder in pediatric patients with an incidence estimated in 1:2,000-4,000 newborns. Significantly, iodide deficiency still remains as the leading cause of hypothyroidism at birth [1]. However, in iodide-sufficient countries, a number of recent studies indicates that 58% to 69% of congenital hypothyroidism is caused by genetic abnormalities resulting in thyroid dysgenesis, including thyroid (hemi)-agenesis, hypoplasia and ectopy. Meanwhile 31% to 42% of the patients have eutopic thyroid gland consistent with genetic defects that impair thyroid hormone synthesis, also called dyshormonogenesis [2]. Other less common genetic causes of congenital hypothyroidism include central hypothyroidism or defects in peripheral thyroid hormone action, cell-membrane transport, or metabolism [3-5].

Hypothyroidism during the first years of life causes stunted growth and irreversible mental retardation when early diagnosis is overlooked [6]. Therefore, neonatal screening programs—one of the major achievements in preventive medicine—primarily seek to detect elevated thyrotropin (TSH) levels, reduced thyroxine (T4) levels, or both at birth allowing a prompt diagnosis and an early onset of levothyroxine therapy, virtually abolishing the development of intellectual disability [7]. Although it does not detect newborns with rare hypothalamic-pituitary-hypothyroidism, determination of TSH levels is considered the most reliable indicator in the early diagnosis of primary congenital hypothyroidism [8]. Recent evidence raised new questions regarding treatment optimization for children with congenital hypothyroidism as Bongers-Schokking et al. [9] demonstrated that overtreatment during early life may lead to low cognitive outcomes, whereas undertreatment, if not complicated by overtreatment, has no significant effects on cognitive development. Although most patients with congenital hypothyroidism develop successfully after optimal levothyroxine replacement, patients with defects of thyroid hormone action, cell-transport, and metabolism do not respond to the classical levothyroxine therapy [10]. Nowadays, the design of new treatment options for patients with these rare conditions is one of the most challenging tasks in the field.

During the last decades, the rising incidence of congenital hypothyroidism consists largely of milder cases with eutopic thyroid gland [11]. However, a relevant clinical question is whether these cases are transient or permanent, thus reinforcing the importance of reassessing the diagnosis at three years of age, when brain development is complete, particularly in patients with low levothyroxine requirements.

Genetics of Congenital Hypothyroidism

In the last two decades, considerable progress has been made in the understanding of the genetic causes of congenital hypothyroidism. Thyroid dysgenesis has been ascribed to mutations in genes responsible for the development or growth of thyroid cells, such as the transcription factors *NKX2-1, FOXE1, PAX8, NKX2-5*, and *GLIS3*, and the TSH receptor (*TSHR)* (Table 1) [12]. However, the genetic mechanisms

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Classification	Gene (OMIM)	Syndrome	Location	Pattern of inheritance	Function
Thyroid dysgenesis	*NKX2-1* (600635)	Choreoathetosis, hypothyroidism, and neonatal respiratory distress	14q13.3	AD	Transcription factors implicated in thyroid gland morphogenesis and maintenance of the thyroid differentiation phenotype.
	FOXE1 (602617)	Bamforth-Lazarus syndrome	9q22.33	AR	
	PAX8 (167415)	Unilateral kidney agenesis	2q14.1	AD	
	NKX2-5 (600584)	-	5q35.1	AD	Transcription factor putatively involved in thyroid gland morphogenesis.
	GLIS3 (610192)	Neonatal diabetes mellitus and hypothyroidism	9p24.2	AR	Transcription factor involved in thyroid gland development. Thyroid hormone resistance?
	CDC8A (609977)	-	1p34.3	AR	Suggested participation in adhesion and migration of thyrocytes.
	TSHR (603372)	-	14q31.1	AR	Receptor for TSH located in the thyroid follicular cell.
Thyroid dyshormonogenesis	*SLC5A5* (601843)	-	19p13.11	AR	Iodide accumulation in the thyroid follicular cell and other tissues.
	TPO (606765)	-	2p25.3	AR	Iodide oxidization and binding to tyrosine residues in thyroglobulin; and coupling of iodotyrosine residues.
	SLC26A4 (605646)	Pendred syndrome	7q22.3	AR	Iodide efflux from the thyroid follicular cell into the colloid.
	TG (188450)	-	8q24.22	AR	Thyroid hormone precursor, storage of iodine and inactive thyroid hormones.
	IYD (612025)	-	6q25.1	AR	Deiodination of iodotyrosines.
	DUOX2 (606759)	-	15q21.1	AR	Hydrogen peroxide generation.
	DUOXA2 (612772)	-	15q21.1	AR	Plasma membrane targeting of DUOX2
Central hypothyroidism	*TSHB* (188540)	-	1p13.2	AR	Beta chain of TSH that confers specificity for TSHR.
	TRHR (188545)	-	8q23.1	AR	Thyrotropin-releasing hormone (TRH) receptor expressed in thyrotropic cells.
	IGSF1 (300137)	Hypothyroidism, short stature and testicular enlargement	Xq26.1	X-linked	Suggested participation in TRH signaling in thyrotropic cells.
	GNAS (139320)	Pseudo-hypoparathyroidism	20q13.32	AD	Alpha subunit of the stimulatory guanine nucleotide-binding protein.
	TBL1X (300196)	Hypothyroidism and hearing loss	Xp22.3	X-linked	Intrinsic component of the thyroid hormone receptor-corepressor complex.
	HESX1 (601802)	Combined pituitary hormone deficiency	3p14.3	AD, AR	Transcription factor essential for development of anterior pituitary cell types.
	LHX3 (600577)		9q34.3	AR	Transcription factor essential for development of anterior pituitary cell types, except corticotropes.
	LHX4 (602146)		1q25.2	AD	Transcription factor involved in the development of the pituitary gland
	SOX3 (313430)		Xq27.1	X-linked	Transcription factor involved in the formation of the hypothalamo-pituitary axis.
	OTX2 (600037)		14q22.3	AD	Transcription factor required for HESX1 gene expression.
	POU1F1 (173110)		3p11.2	AD, AR	Pituitary-specific transcription factors essential for development of anterior pituitary cell types.
	PROP1 (601538)		5q35.3	AD, AR	
Peripheral hypothyroidism	*THRB* (190160)	Refetoff syndrome	3p24.2	AD	Thyroid hormone receptors
	THRA (190120)	-	17q21.1	AD	
	SLC16A2 (300095)	Allan-Herndon-Dudley syndrome	Xq13.2	X-linked	Thyroid hormone cell-membrane transporter. Highly relevant for T3 accumulation in neurons.
	SECISBP2 (607693)	-	9q22.2	AR	Binding to selenocysteine insertion sequences mRNA elements to decode UGA codons as selenocysteines.

Table 1: Classification and genetic etiology of congenital hypothyroidism.

underlying defects in thyroid development for the vast majority of the cases remain unknown, as the genetic causes of thyroid dysgenesis were only identified in 5% to 10% of the patients [12]. Significantly, thyroid dysgenesis caused by genetic defects in transcription factors is usually associated with congenital defects in other organs, such as brain, lung, kidney, and pancreas [13]. However, the majority of patients with thyroid dysgenesis do not show syndromic types of congenital hypothyroidism suggesting the presence of molecular defects in unidentified genes. Accumulating evidence supports that the genetics of thyroid dysgenesis may follow non-Mendelian modes of inheritance [14].

Congenital hypothyroidism due to thyroid dyshormonogenesis is a heterogeneous disorder caused by mutations in any of the genes involved in the biosynthesis of thyroid hormones, such as the sodium/iodide symporter (*SLC5A5*), pendrin (*SLC26A4*), thyroid peroxidase *(TPO*), dual oxidase 2 (*DUOX2*), dual oxidase maturation factor 2 (*DUOXA2*), iodotyrosine dehalogenase 1 (*IYD*), and thyroglobulin *(TG*) (Table 1) [15]. These mutations produce a diverse spectrum of phenotypes ranging from subclinical to severe hypothyroidism [16]. Particularly, mutations in *SLC26A4* gene causes syndromic hypothyroidism characterized by moderate hypothyroidism—usually associated with goiter—and sensorineural hearing loss [17]. Muzza et al. [18] showed that congenital hypothyroidism due to *DUOX2* mutations is permanent in 65% of cases but transient in 35%; consistent with this observation *DUOX2* mutations may result in a clinical spectrum of disease ranging from congenital hypothyroidism to euthyroid goiter in adulthood.

Central hypothyroidism is rarely caused by genetic defects in the beta chain of TSH (*TSHB)*, TSH-releasing hormone receptor (*TRHR*), and immunoglobulin superfamily member 1 (*IGSF1*) causing isolated TSH deficiency, or the alpha subunit of the stimulatory guanine nucleotide-binding protein (*GNAS*) resulting in defective TSH signaling (Table 1). In most cases of central hypothyroidism, however, TSH deficiency is associated with mutations in transcription factors involved in pituitary gland development, such as *HESX1, LHX3, LHX4, SOX3, OTX2, POU1F1* and *PROP1* causing other pituitary hormone deficiencies, as part of congenital hypopituitarism (Table 1) [3]. Very recently, mutations in transducing beta-like protein 1 (*TBL1X*) gene were associated with central hypothyroidism and hearing loss (Table 1) [19].

Peripheral hypothyroidism is usually associated with peripheral resistance to the action of thyroid hormone. Indeed 80% of these cases harbor dominantly inherited mutations in the gene encoding the thyroid hormone receptor beta *(THRB)* (Table 1) [4]. Recently, mutations were also reported in the gene encoding thyroid hormone receptor alpha *(THRA)* (Table 1) [4]. Additionally, peripheral hypothyroidism may also be caused by defects in cell-membrane thyroid hormone transport, such as in Allan-Herndon-Dudley syndrome where mutations in monocarboxylate transporter 8 *(SLC16A2)* gene were reported [5], and thyroid hormone metabolism defects due to mutations in the gene selenocysteine insertion sequence binding protein 2 (*SECISBP2*) (Table 1) [5].

Thyroid hormone serum transport defects constitute a group of genetic abnormalities that affect each of the three major thyroid hormone transport proteins—thyroxine-binding globulin, transthyretin and albumin [20]. Mutations in thyroid hormone transport proteins reduce thyroid hormone concentration in serum, but do not alter the metabolic state or cause thyroid disease. However, early recognition of these conditions is important to prevent unnecessary therapy with possible untoward effects.

Epigenetic Mechanisms Involved in Congenital Hypothyroidism

Discordance of monozygotic twins for thyroid dysgenesis suggests that epigenetic mechanisms may underlie abnormal thyroid morphogenesis [14]. Abu-Khudir et al. [21] compared the transcriptome and methylome of eutopic and ectopic thyroid tissues. Although the thyroid tissues showed a different gene expression pattern, the expression profile was independent of the methylation status of gene promoters. However, bisulfite sequencing showed tissue-specific differentially methylated regions within the *FOXE1* gene promoter between thyroid tissues (either eutopic or ectopic) and matched leukocytes. Functional analysis revealed that methylation represses *FOXE1* gene expression suggesting a possible epigenetic control of thyroid development [22].

Pseudohypoparathyroidism is a group of disorders characterized by resistance to the parathyroid hormone. Mutations in *GNAS* gene causes pseudohypoparathyroidism type 1A leading to multihormone resistance syndrome, including TSH resistance. While epigenetic changes in the *GNAS* locus, initially described in parathyroid hormone isolated resistance, causes pseudohypoparathyroidism type 1B. Nonetheless, Romanet et al. [23] reported epigenetic changes in *GNAS* locus in a patient with parathyroid hormone resistance, congenital hypothyroidism, and macroglosia. Significantly, this case report added a new cause for the etiologic diagnosis of congenital hypothyroidism involving epigenetic mechanisms.

Haploinsufficiency of euchromatin histone methyltransferase 1 (*EHMT1*) was found responsible for 9q34.3 subtelomeric deletion syndrome. Particularly, individuals with large deletions within 9q34.3 display hypothyroidism and brain anomalies [24], suggesting that aberrant *EHMT1* expression might also be associated with congenital hypothyroidism. Strikingly, the heterodimer complex EHMT1/2 maintains DNA methylation via recruitment of DNA methyltransferase [25]. Thus abnormal *EHMT1* expression might cause aberrant DNA methylation patterns and eventually lead to thyroid dysfunction. Future investigation regarding EHMT1/2 complex levels and its associated epigenetic modifications in patients with congenital hypothyroidism might create a new direction for the identification of abnormal epigenetic mechanisms involved in the disease.

Diagnostic Studies to Determine the Underlying Etiology

Diagnosis and treatment follow-up of congenital hypothyroidism is based on serum thyroid function tests including TSH and either free T4 or total T4 combined with some measure of binding proteins, such as a T3 resin uptake. However, other diagnostic studies—thyroid ultrasonography, thyroid scintigraphy using ^{123}I-iodide or ^{99m}Tc-pertechnectate, saliva/serum iodide ratio, perchlorate discharge test, and dosage of TG, TSH receptor blocking antibodies, and urinary iodine—may be used to determine the underlying etiology of the disease. Although these diagnostic studies generally do not alter the treatment decision, and sometimes they are considered unnecessary, may lead to a specific genetic test to confirm the underlying etiology, particularly in patients with thyroid dysgenesis and dyshormonogenesis. Specifically, patients with iodide transport defect due to mutations in *SLC5A5* gene show thyroid gland enlargement, normal to increased serum TG levels and severely reduced to absent ^{123}I-iodide or ^{99m}Tc-pertechnectate accumulation in the thyroid gland [26,27].

Molecular Diagnosis of Congenital Hypothyroidism

Current consensus guidelines from the European Society of

Paediatric Endocrinology on screening, diagnosis and management of congenital hypothyroidism state clear recommendations regarding genetic counseling of affected patients and their families [28]. However, the prevalence of identifiable disease-causing mutations is rather low and many questions regarding the molecular pathogenesis of the disease remain unsolved. A meta-analysis demonstrated that only 5% to 10% of patients with thyroid dysgenesis and 50% to 90% of patients with thyroid dyshormonogenesis showed a monogenic disorder when studied at the molecular level using PCR-based techniques followed by Sanger sequencing focusing on target genes [29].

Targeted next-generation sequencing allows a low-cost sequencing of multiple target genes in simultaneous, constituting an attractive alternative to diagnose prevalent endocrine genetic disorders, such as congenital hypothyroidism [30]. Very recently, three independent reports evaluated the clinical usefulness of targeted next-generation sequencing in the diagnosis of congenital hypothyroidism [31-33]. In spite of significant effort, still considerable proportions of patients (18% to 36%) remain undiagnosed suggesting the existence of novel genetic etiologies that remain to be elucidated.

The introduction of targeted next-generation sequencing has led to the identification of novel monoallelic or biallelic mutations in the *DUOX2, TPO, TG, TSHR*, and *PAX8* genes in large cohorts of Chinese patients with transient or permanent congenital hypothyroidism [34-37]. Interestingly, the analysis revealed that multiple mutations in different genes involved in congenital hypothyroidism coexist within a single patient. Of note, although most patients with mutations in the *TSHR* gene showed normal size thyroid glands, while few patients had hypoplastic glands, Fu et al. [37] reported a patient with combined *TSHR* and *DUOX2* mutations showing an increased thyroid gland size. Thus, suggesting that the variability of phenotypes observed by thyroid ultrasonography may be the result of an uncharacterized array of multiple genetic defects. Further details regarding the identification of multiple mutations in different genes involved in thyroid hormone biosynthesis have been recently reviewed [38]. Altogether, these advances in the molecular diagnosis expand the landscape of mutations associated with congenital hypothyroidism and, along with environmental factor, may provide a novel explanation for the variability in genotype and phenotype correlations observed in patients with congenital hypothyroidism.

Whole-exome sequencing has emerged as a powerful high-throughput DNA sequencing technology to elucidate genetic defects underlying rare human disorders of unknown etiology, aiding the discovery of novel disease genes [39]. In the thyroid field, whole-exome sequencing analysis revealed loss-of-function mutations in the *IGSF1* gene in patients with central hypothyroidism—secondary to a reduced TRH-signaling in the pituitary—associated with short stature and testicular enlargement [40], and an unexpected homozygous missense mutation in the *SLC26A4* gene, which had previously been associated with a thyroid dyshormonogenesis phenotype, in a consanguineous family with thyroid hypoplasia [41]. Very recently, whole-exome sequencing analysis of a consanguineous family with thyroid dysgenesis revealed a novel homozygous missense mutation in the *CDC8A* gene (Table 1) [42]. In light of the potential of whole-exome sequencing, future studies will open new avenues in the genetics of congenital hypothyroidism, particularly when evaluating patients with thyroid dysgenesis.

Significance of Molecular Diagnosis in Congenital Hypothyroidism

Although the uncertainty in the molecular etiology of congenital hypothyroidism has not been a significant concern as management of the disease is mainly based on restoring thyroid function. However, accumulated knowledge regarding the etiology of the disease has significant implications for genetic counseling to the family of patients. Particularly, genetic analysis provides the ultimate diagnostic confirmation and correct counselling of the family as well as early focused support for affected patients. Based on current outcome data, an immediate adequate replacement therapy should be initiated and maintained throughout the lifespan for most patients.

The identification of the molecular cause of congenital hypothyroidism allows the prediction of syndromic hypothyroidism and health care support for affected patients. Particularly, the identification of mutations in transcription factors associated with thyroid dysgenesis implies that special attention should be paid to neurological development, lung disease, and renal malformations. The identification of *GNAS* and *GLIS3* mutations should lead the endocrinologist to focus on the early diagnosis of pseudohypoparathyroidism and diabetes mellitus, respectively. Significantly, levothyroxine therapy in patients with thyroid dyshormonogenesis should be carefully monitored in order to reduce TSH levels below the normal range to prevent the risk of developing thyroid cancer within the goiter in adulthood [43,44].

Muzza et al. [18] reported that 7 out of 11 (64%) patients with congenital hypothyroidism due to mutations in *DUOX2* gene showed normal TSH levels during newborn screening. This suggests the importance of repeating thyroid function evaluation in patients at risk of *DUOX2* defects, particularly younger siblings of patients with known thyroid dyshormonogenesis. Additionally, early genetic diagnosis in patients with congenital hypothyroidism will allow subsequent preclinical diagnoses of younger siblings within the same family as patients with delayed onset on clinical hypothyroidism had already signs of developmental delay at time of diagnosis [26]. Moreover, the identification of mutations in the *TG* and *TPO* genes—usually associated with dyshormonogenic goiter—in pediatric patients indicates that in future pregnancies of their mothers the presence of prenatal goiter due to a defect in thyroid hormone biosynthesis should be carefully monitored. Such goiter can be detected by fetal ultrasound and treated with the administration of intra-amniotic levothyroxine to prevent goiter-associated dystocia and improve the fetal neurodevelopment [16].

In the presence of central congenital hypothyroidism, genetic diagnosis of the hypothalamo–pituitary axis is indicated as the majority of patients may develop additional pituitary hormone deficiencies. Patients with mutations in pituitary transcription factors require long-term surveillance for evolving pituitary hormone deficiencies underlying significant risks, such as life-threatening hypoglycaemia. Conversely, genetic defects causing isolated TSH deficiency enables reassurance that additional hormone deficits will not develop. Significantly, follow-up studies of families with *TRHR* or *IGSF1* mutations suggest that family screening following molecular diagnosis of a proband may identify apparently healthy siblings with hitherto undiagnosed central hypothyroidism [3].

Regarding treatment options, iodide supplementation can ameliorate thyroid function in patients with reduced, although not absent, iodide accumulation due to mutations in *SLC5A5* gene as well as in patients with mutations in *IYD* gene and, therefore, should be considered, either as adjunct or alternative levothyroxine replacement therapy [45]. Additionally, early genetic diagnosis of patients with impaired sensitivity to thyroid hormone is important to avoid misdiagnosis as the majority of individuals with thyroid hormone resistance, if left alone, adequately compensate for the defective receptor through increased

thyroid hormone secretion. Moreover, early diagnosis in patients with Allan-Herndon-Dudley Syndrome due to mutations in *SLC16A2* gene may allow the possibility to enter into clinical trials to assess the effect of thyroid hormone analogs—triiodothyroacetic acid *(*TRIAC*)* or diiodothyropropionic acid *(*DITPA)—as no adequate treatment is currently available [46].

Recently, Mizokami et al. [47] demonstrated severely reduced iodide concentration in the breast milk of a women diagnosed with congenital hypothyroidism due to a mutation in the *SLC5A5* gene; thus highlighting the importance of genetic screening for *SLC5A5* gene mutations to prevent the development of hypothyroidism in breast-fed newborns due to iodide-deficient breast milk. Prophylactic iodine supplementation is essential for such newborns in order to prevent mental retardation.

Concluding Remarks

The genetics of congenital hypothyroidism needs to be further elucidated, particularly the identification of defective genes involved in thyroid dysgenesis. Significantly, in the last few years, many reports described the identification of novel genes involved in the organogenesis of the thyroid tissue, such as *EYA, HHEX, HOXB3, HOXD3*, *HOXD3, ISL, SHH, HES1* and *NTN1* [48-50] where mutations involved in thyroid pathology have not yet been reported.

Genetic screening should be considered in all pediatric patients with congenital hypothyroidism. After identification in neonatal screening, the biochemical confirmatory diagnosis of the disease should be followed by diagnostic studies to rule out transitory congenital hypothyroidism and to evaluate thyroid function in detail to obtain insights into the underlying etiology of the disease, particularly when PCR-based approaches are used to evaluate the sequence of individual genes.

The introduction of next-generation sequencing platforms will probably allow a major change in the traditional diagnosis and understanding of the molecular bases of congenital hypothyroidism. First, the efficient screening of gene panels to assess inherited mutations in multiple genes involved in congenital hypothyroidism will increase the diagnostic efficiency. Second, the identification of the coexistence of multiple mutations in the same gene or in different genes involved in the etiology of the disease could contribute to a deeper understanding of the correlation between genotype and phenotype. Third, the massive identification of novel disease-causing gene mutations will be of great importance to gain insights into the etiology of the disease, allowing further optimization of gene panels to screen for the molecular diagnosis of the disease.

Future research should aim to enhance our knowledge of the pathophysiology of congenital hypothyroidism and to determine whether the identification of the molecular defect including the coexistence of genetic and epigenetic deficiencies will help us to improve patient care and the therapeutic outcomes.

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