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REVIEW

New genetics in congenital hypothyroidism

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Abstract

Introduction Congenital hypothyroidism (CH) is the most frequent neonatal endocrine disorder and one of the most common preventable forms of mental retardation worldwide. CH is due to thyroid development or thyroid function defects (primary) or may be of hypothalamic-pituitary origin (central). Primary CH is caused essentially by abnormal thyroid gland morphogenesis (thyroid dysgenesis, TD) or defective thyroid hormone synthesis (dyshormonogenesis, DH). TD accounts for about 65% of CH, however a genetic cause is identified in less than 5% of patients.

Purpose The pathogenesis of CH is largely unknown and may include the contribution of individual and environmental factors. During the last years, detailed phenotypic description of patients, next-generation sequence technologies and use of animal models allowed the discovery of novel candidate genes in thyroid development, function and pathways.

Results and conclusion We provide an overview of recent genetic causes of primary and central CH. In addition, mode of inheritance and the oligogenic model of CH are discussed.

Keywords Congenital hypothyroidism · Genetics · Thyroid dysgenesis · Dyshormonogenesis · Oligogenism · Epigenetics

Introduction

Congenital hypothyroidism (CH) is the most common neonatal endocrine disorder, affecting about 1:3000 newborns worldwide and one of the most preventable causes of motor and cognitive disorders. CH may be caused by abnormal development or function of the thyroid gland (primary CH), by hypothalamus and pituitary disorders (central CH (ceCH)), but also due to impaired thyroid hormone action. During the last years, the use of modern and faster genetic approaches, such as next-generation sequencing techniques (NGS; whole-exome sequencing, WES), has provided new insights into the genetics of CH. Thanks to advance in genetic testing and detailed phenotypic description of patients and/or families with CH that new candidate genes have been identified. Furthermore, they allowed to extend the assumed thyroid phenotype resulting from mutations in known genes responsible essentially for thyroid hormone synthesis, causing dyshormonogenesis (DH). We present in this review an overview on novel genes on CH and proposed modes of inheritance of this complex disease.

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Novel genes in primary CH

Thyroid dysgenesis (TD)

TD due to thyroid development disorder is the most frequent cause of permanent primary CH, explaining ~65% of CH cases [1, 2]. The clinical phenotype varies from absence of thyroid gland (athyreosis), severe hypoplasia, to ectopy or hemiagenesis of the thyroid. In contrast to TD with athyreosis or thyroid ectopy, the other 35% is best described as gland in situ (GIS). A genetic cause is identified in less than 5% of TD, including mutations in TSH receptor (*TSHR)* [3] or in genes encoding transcription factors involved in thyroid development (*TTF1/NKX2-1, PAX8, FOXE1, NKX2-5, GLIS3*) [4, 5]. Based on thyroid phenotype of transgenic animal models, the implication of these genes has been validated by mutational screening in cohorts of patients with syndromic or no CH. We describe here the novel candidate genes identified during the last 5 years in CH due to TD.

CDCA8/BOREALIN (OMIM #609977)

By WES in familial TD cases, Carré et al. found Borealin (encoded by *BOREALIN/CDCA8*) to be also involved in thyrocyte migration and adhesion, explaining cases of thyroid ectopy [6]. Borealin is a major component of the chromosomal passenger complex, which has well-described functions in chromosome segregation and cytokinesis. It is highly expressed during embryogenesis. *BOREALIN* biallelic mutations have been identified in a consanguineous family and two distinct monoallelic mutations in CH sporadic cases. Patients harboring mutations had athyreosis ($n = 1$), and ectopy ($n = 2$), while several family members showed normal thyroid function and hemiagenesis/asymmetrical thyroid ($n = 3$) or nodules ($n = 2$), and one patient also suffered from papillary thyroid cancer. It remains unclear whether nodules and papillary thyroid cancer could be a long-term cause of *BOREALIN* mutations. This work provided evidence for: (1) *BOREALIN* expression in the human embryonic thyroid gland in thyrocytes before and after onset of thyroid hormone synthesis (8 and 12 gestational weeks) in contrast to very low expression in adult thyroid tissue, and (2) BOREALIN involvement in the process of migration and adhesion of human thyrocytes, in accordance with ectopy as essential thyroid phenotype.

NTN1/Netrin1 (OMIM #601614)

NTN1 encodes for netrin1, which is part of a family of laminin-related secreted proteins, involved in cell migration and survival during development and adulthood and possibly in the development of pharyngeal vessels. Netrin1 acts as an axon guidance molecule during neural development and mutations have been identified in patient with congenital mirror movements [7]. One patient with congenital heart defect and TD (ectopy) has been described with deletion in *NTN1* [8]. Study of ntn1-deficient zebrafish embryos showed defective aortic arch artery formation and abnormal thyroid morphogenesis resulting from a lack of proper guidance exerted by the dysplastic vasculature in these embryos. The exact role of NTN1 and its implication on thyroid and cardiac congenital defects remain to be confirmed.

JAG1 (OMIM #601920)

JAG1 encodes for jagged1 protein, in the Notch pathway, and heterozygous mutations have been described in the Alagille syndrome, a rare multisystemic developmental disorder. The Notch pathway is implicated in thyroid development and hypothyroidism appears in zebrafish when interruption of the Notch signaling [9, 10].

The thyroid function in 21 patients harboring *JAG1* mutations has been assessed and *JAG1* has been also screened in an Italian cohort of 100 patients with TD [10]. The authors reported a prevalence of hypothyroidism in 6/21 patients with Alagille syndrome, 2 of them with thyroid hypoplasia, while 2 *JAG1* variants in the heterozygous state were found in 4/100 CH cases (3 with TD, 2 with cardiac malformations). These data may suggest a role for *JAG1* in the pathogenesis of TD, mainly thyroid hypoplasia.

TUBB1 (OMIM #612901)

Mutations in the *TUBB1* (tubulin, beta 1 class VI) gene have recently been identified in patients from three families with TD (mostly ectopy) and abnormal platelet physiology [11]. Using WES, a homozygous missense mutation in *TUBB1* has been found in two siblings of a consanguineous family with TD. By direct *TUBB1* sequencing in a cohort of 270 patients with TD, two more mutations have been identified in two distinct families with TD. TUBB1 encodes for a member of the b-tubulin protein family. B-tubulins are one of two core protein families that heterodimerize to form a/b-tubulin dimers, which assemble into microtubules, one of the major cytoskeletal structures. All three *TUBB1* mutations led to nonfunctional a/b-tubulin dimers that cannot be incorporated into microtubules. *Tubb1* expression was shown in the developing and adult thyroid in humans and mice. The *Tubb1* knock-out mice have large platelets and show hypothyroidism, in accordance with the phenotype in mutated patients. Transgenic mice thyroids exhibited proliferation defects during early development (embryonic day E9.5), altered migration at E11.5 and E13.5, and failure of hormone secretion at E17.5 and

Table 1 Genes associated with thyroid dysgenesis or syndromic primary CH

Gene (OMIM)	Protein role	Typical thyroid phenotype	Mode of inheritance	Associated conditions
TSHR (603372) [3]	GPCR	Complete or partial TSH resistance: apparent athyreosis → GIS and severe → mild hypothyroidism	AD/AR	
NKX2-1 (600635)	NF	Variable	AD	Respiratory distress, choreoathetosis
FOXE1 (602617)	NF	Athyreosis, severe hypoplasia	AR	Cleft palate, choanal atresia, spiky hair
PAX8 (167415)	NF	Variable	AD	Urogenital tract defects
NKX2-5 (600584)	NF	GIS, variable hypothyroidism	Unclear	Congenital heart malformations
GLIS3 (610192)	NF	GIS	AR	Neonatal diabetes, polycystic kidneys, cholestasis
JAG1 (601920) [9, 10]	Jagged 1: Notch receptor ligand	Variable orthotopic hypoplasia	AD	Heart malformations, liver cholestasis
TBX1 (602054)	NF	GIS	AD	Di George syndrome with congenital heart malformations
NTN1 (601614) [8]	Laminin-related secreted protein	Thyroid ectopy	Unknown	Arthrogryposis
BOREALIN (609977) [6]	Cell division cycle associated protein 8 or Borealin: component of the chromosomal passenger complex	Thyroid ectopy, hemiagenesis, thyroid asymmetry	AD/AR	None in sporadic cases
TUBB1 (612901) [11]	Member of the β-tubulin protein family	Thyroid dysgenesis	AD	Formation of macroplatelets and hyperaggregation of platelets

NF nuclear factor, *GPCR* G protein-coupled receptor, *AD* autosomal dominant, *AR* autosomal recessive, *GIS* gland in situ

adulthood. All these complex mechanisms require proper microtubule function. Interestingly, two of the novel TUBB1 mutations were associated with basal activation and exaggerated aggregation of platelets.

Syndromic CH with TD Patients with CH due to TD or GIS may have extrathyroidal-related abnormalities. The most common forms of syndromic hypothyroidism with TD include Bamforth–Lazarus syndrome (*FOXE1*) and the brain–lung–thyroid syndrome (*NKX2-1*). Ongoing genetic research has identified candidate genes associated with syndromic CH, such as *TBX1* (di George syndrome), *SALL1* (Townes–Brocks syndrome), *URB1* (Johanson–Blizzard syndrome), *ELN* and *BAZ1B* (Williams-Beuren syndrome), *KMT2D* and *KDM6A* (Kabuki syndrome), and *KAT6B* (Ohdo syndrome, Genitopatellar syndrome).

Neonates and infants with Down syndrome display often mild, nonautoimmune subclinical hypothyroidism or primary CH. Underlying mechanism of thyroid dysfunction remains poorly understood. New insights were provided by a study in Dyrk1A(+/++) mice showing abnormal thyroid development and function [12]. Transgenic (Dyrk1a+/++) mice have been studied, as they have neuronal, synaptic, learning, and memory impairments similar to those found in Down syndrome. Mice had larger primary thyroids surface at an early developmental stage, (embryonic day E15.5), with lower T4 levels and smaller thyroglobulin (*TG*) differentiated surface areas, a deregulation of transcription factors involved in thyroid development, and abnormal thyroid function and morphology. The thyroids at adult ages in these mice were disorganized with large regions of small follicles. These abnormalities were similar to those found in thyroids from human Down syndrome affected fetuses. *DYRK1A*, a gene in the Down syndrome critical region, is a candidate gene involved in TD.

Table 1 summarizes genes associated with TD.

Thyroid DH

Defects in any of the proteins indispensable for thyroid hormone synthesis lead to DH. In these patients, thyroid development and differentiation is normal; however, one of the critical steps of the synthetic machinery of the thyroid follicular cells is impaired. These include defects in (1) iodine uptake into thyroid follicular cells due to mutations in the sodium/iodide symporter (*NIS/SLC5A5*); (2) organification defects due to mutations in the *TPO*, *DUOX2*, and *DUOXA2* genes constituting the peroxidase enzyme system, or mutations in the *Pendrin* gene (*SLC26A4*); (3) defects in *TG* synthesis, storage, or release; and (4) defective IYD (*DEHAL1*) activity leading to failure of thyroid follicular cell iodide recycling [13]. In contrast to TD, DH is inherited in an autosomal recessive pattern and CH is isolated in most

Table 2 Genes associated with thyroid dyshormonogenesis

Gene (OMIM)	Protein role	Typical thyroid phenotype	Associated conditions and mode of inheritance
GNAS (139320)	Alpha subunit of the stimulatory guanine nucleotide-binding protein (G protein)	Partial TSH resistance, mild hypothyroidism	Pseudohypoparathyroidism (PHP, multiple hormone resistances) of maternal inheritance, parental imprinting of gene locus
SLC5A5 (601843)	NIS: sodium–Iodide symporter	Absent or low iodide uptake at scintigraphy, variable hypothyroidism, goiter	AR
SLC26A4/PDS (605646)	Pendrin: anion transporter	PIOD, mild-to-moderate hypothyroidism, goiter	Pendred syndrome: sensorineural deafness with enlarged vestibular aqueduct (EVA), high serum Tg, predisposition to alkalosis, AR
DUOX1/DUOX2 (606758/606759) [20]	Dual oxydases: peroxide generating system	PIOD or CIOD, goiter, transient or permanent hypothyroidism of variable severity	High serum Tg, AR/AD
DUOXA2 (612772)	Dual oxydase associated protein: endoplasmic reticulum chaperone protein	PIOD or CIOD, goiter, transient or permanent hypothyroidism of variable severity	High serum Tg, AR
TPO (606765)	Thyroid peroxidase: iodide organification and thyronine coupling	CIOD, severe hypothyroidism, goiter	High serum Tg, AR
TG (188450)	Thyroglobulin: glycoprotein precursor to the thyroid hormones	High iodide uptake, variable hypothyroidism, congenital or rapidly growing goiter	Low serum Tg, AR
IYD/DEHAL (612025)	Dehalogenase providing iodide salvage in thyroid)	Conserved iodide uptake, negative perchlorate discharge test, goiter, variable hypothyroidism	High serum Tg and MIT/DIT concentrations in serum and urine, AR (dominant inheritance of goiter with incomplete penetrance)
SLC26A7 (608479) [14, 15]	Anion transporter	Goiter, variable hypothyroidism, conserved iodide uptake, partial defect at perchlorate discharge	High serum Tg, AR

Tg thyroglobulin, *AD* autosomal dominant, *AR* autosomal recessive, *PIOD* partial iodide organification defect, *CIOD* complete iodide organification defect, *MIT* monoiodotyrosine, *DIT* diiodotyrosine

cases. Pendred syndrome, due to mutations in *SLC26A4*, is the exception with patients showing sensorineural hearing loss, enlarged vestibular aqueduct, and usually goiter.

SLC26A7 (OMIM #608479)

Biallelic mutations in *SLC26A7* have recently been reported to cause goitrous CH [14, 15]. SLC26A7 is a member of the same transporter family as SLC26A4 (pendrin) an anion exchanger with affinity for iodide and chloride. However, in contrast to pendrin, SLC26A7 does not mediate cellular iodide efflux and affected individuals have normal hearing [14, 15].

New thyroid phenotypes in known genes Mutations in *SLC26A4* [16], *DUOX2* [17], and *TPO* [18] have been unexpectedly found in patients with nongoitrous CH and thyroid hypoplasia, narrowing the gap between TD and DH. Recently, *DUOX2* mutations have also been reported in patients with thyroid ectopy; however, further studies are needed to confirm and explain this phenotype [19]. The first CH patients bearing both *DUOX1* as well as *DUOX2* mutations have been reported, suggesting a possible digenic cause of CH [20]. Table 2 resumes genes associated with DH.

Novel genes in ceCH

ceCH can be isolated or in the context of multiple pituitary hormone deficiency (MPHD). The number of probable genetic causes of isolated and combined/syndromic ceCH has increased due to advances in molecular testing, as in primary CH.

CeCH is more frequently part of MPHD and can be associated with one or more other pituitary hormone deficiencies. In addition, a certain percentage of affected patients have morphological abnormalities of the pituitary gland or hypothalamus, or other neurological defects [21, 22].

Concerning isolated CeCH, it is mainly due to biallelic mutations of *TSHβ* [23, 24] and *TRHR* [25], the last one described in only a few families. We present below novel genes identified last years in patients with ceCH.

IGSF1 (OMIM #300137)

Immunoglobulin superfamily member 1 gene (*IGSF1*) mutations are the molecular cause of an X-linked syndrome including mild-to-moderate ceCH. Associated features include abnormal testicular growth leading to adult macroorchidism, a tendency toward pubertal delay, low prolactin level, and sometimes, reversible growth hormone deficiency [26]. Some female carriers can have altered thyroid function. Recent data indicate IGSF1 as the most frequently implicated gene in congenital ceCH [27].

TBL1X (OMIM #300196)

Mutations in the *TBL1X* gene are a second cause responsible for X-linked ceCH. TBL1X, transducin-like protein 1, is an essential subunit of the nuclear receptor corepressor (NCoR)-silencing mediator for retinoid and thyroid hormone receptors (SMTRs) complex, the major thyroid hormone receptor (TR) CoR involved in T3-regulated gene expression. Hearing loss is often an additional clinical feature [28].

IRS4 (OMIM #300904)

Recently, mutations in *IRS4* have also been identified in X-linked ceCH in five families. The insulin receptor substrate (IRS) family acts as interface between tyrosine kinase receptors, including the insulin, leptin, and insulin-like growth factor 1 (IGF-1) receptors, and multiple intracellular signaling pathways. Since IRS4 is involved in leptin signaling, the proposed mechanism of ceCH may be disrupted leptin signaling [29]. Table 3 shows genes implicated in central hypothyroidism.

Mode of inheritance

The pathogenesis of CH is largely unknown and may include the contribution of individual and environmental factors. Although CH is typically reported as sporadic, several findings in humans and experimental models support a relevant genetic origin. In favor of genetic origin is the frequent findings of thyroid abnormalities in first degree relatives of CH cases [30] and the increased incidence of CH in consanguineous and in specific ethnic groups [31]. Moreover, CH is associated with a 20-fold increased risk of having other congenital abnormalities [32] and several syndromes have been associated with variable thyroid defects, such as Alagille, di George, Williams-Beuren, Kabuki, and Genitopatellar syndromes.

Until recently, the investigations on CH pathogenesis with classical screening methods (e.g., Sanger sequencing) reported a poor rate of positive results. In particular, monogenic defects had been described in rare familial settings, mainly associated with biallelic inactivating mutations in genes involved in hormonogenesis [33]. On the other hand, heterozygous mutations in thyroid transcription factor genes have been identified in less than 5% of cases with TD, associated with variable penetrance and expressivity of thyroid defects [33]. These data suggested the involvement of still unidentified genes or the contribution of alternative mechanisms. Nevertheless, recent findings support a more significant role for genetic etiology in TD. In particular, a French National Survey reported an incidence of CH and of thyroid developmental abnormalities higher than expected among relatives of TD cases [30]. Therefore, CH can be considered a disease with a strong genetic component, but with a largely missing explanation for its heritability. The continuous discovery of novel candidate genes and pathways involved in thyroid development, by means of NGS technologies and by exploring animal models for thyroid development, provides new clues in support of a heritable origin of CH [10, 15, 18]. Of note, recent targeted NGS studies in CH cohorts detected the presence of heritable variations in more than half of CH patients [34, 35]. This approach has also demonstrated the existence of an overlap of genetic aetiologies in TD and DH; indeed, mutations in genes characteristically associated with TD were detected in cases with GIS and vice-versa [35]. This finding may explain the low mutational detection rate reported by the classic phenotypical approaches.

Oligogenic origin

The possibility of a multigenic origin of CH was initially supported by mouse models [36]. In particular, the double heterozygous mice for *PAX8/TTF1* (now called *NKX2-1)* (DHPT mice) were variably showing a euthyroid or CH phenotype. The authors noticed that the hypothyroid phenotype was always present when the DHPT were derived from a particular strain (B6 mice) in contrast with those from the SV strain, which were invariably euthyroid. After in-depth genetic investigations, they found B6 strain to carry a polymorphic missense variant in a gene that was found to be expressed in the developing thyroid (*Dnajc17*) and showed that the polymorphic *Dnajc17* allele was less potent than wild-type one on a transcription assay [36].

NGS technologies, allowing the simultaneous analysis of multiple genes, have subsequently confirmed the role of oligogenic inheritance of human CH. In particular, a target sequencing analysis of 11 CH-associated genes have been performed in 177 Italian patients with different CH subtypes, demonstrating the presence of a likely pathogenic variant in more than one gene in 25% of cases, associated with both eutopic GIS and TD [35]. The oligogenic origin of CH was confirmed by the cosegregation of variants with CH or nonautoimmune hypothyroid state in several pedigrees.

These results indicated that the pathogenesis of CH can frequently be due to the sum of alterations lying in rare polymorphic alleles, even acting at different levels (thyroid morphogenesis or hormonogenesis), with a modest functional impairment on thyroid function when expressed alone. This observation might be a possible explanation for the apparently sporadic occurrence of CH, and for the variable expressivity and penetrance of causal mutation

Table 3 Genes associated with central CH and related phenotypes

	Gene (OMIM*)	Protein function	Phenotype	Inheritance
Isolated central CH	*TSHβ* (188540) [23, 24]	Hormone subunit	Neonatal onset with low TSH, high αGSU, and normal PRL serum levels, pituitary hyperplasia reversible on L-T4	AR
	TRHR (188545) [25]	GPCR	Normal TSH and low PRL serum levels, blunted TSH/PRL responses to TRH	AR
	TBL1X (300196) [28]	NF	Mild isolated central CH in males with normal TSH serum levels and normal response to TRH stimulation test; associated hearing defects	X-linked
	IRS4 (300904) [29]	NF	Mild isolated central CH in males with normal TSH serum levels, blunted TSH response to TRH	X-linked
Multiple pituitary hormone deficiencies	*IGSF1* (300137) [26, 27]	Plasma membrane protein of unresolved function	Normal TSH serum levels and blunted response to TRH test; low PRL levels, variable GH deficiency, transient mild hypocortisolism, and metabolic syndrome; post-pubertal macrorchidism	X-linked
	PROP1 (601538)	NF	Variable age of onset, combined with GH, PRL LH/FSH deficiencies, and delayed ACTH defects, small-to-large pituitary volume	AR
	POU1F1 (173110)	NF	Variable age of onset, associated with GH and PRL deficiency, prominent forehead, midface hypoplasia, depressed nose	AR, AD
	HESX1 (601802)	NF	Hypopituitarism associated with septo-optic dysplasia	AR, AD
	SOX3 (313430)	NF	Anterior pituitary hypoplasia with ectopic posterior pituitary, persistent craniopharyngeal canal and learning difficulties	X-linked
	OTX2 (600037)	NF	Anterior pituitary hypoplasia with ectopic posterior pituitary and ocular defects (ano-/micro-ophthalmia/retinal dystrophy)	AD
	LHX3 (600577)	NF	Hypopituitarism with inconstant ACTH defect, variable pituitary gland volume, short and rigid cervical spine and variable hearing defect	AR
	LHX4 (602146)	NF	Variable hypopituitarism, anterior pituitary hypoplasia with ectopic posterior pituitary, Arnold–Chiari syndrome, hypoplasia of the corpus callosum	AR, AD
	LEPR (601007)	Cytokine receptor	Hyperphagia, obesity, central hypogonadism	AR
	SOX2 (184429)	NF	Variable hypopituitarism, pituitary hypoplasia, microphthalmia, variable learning difficulties	AD
Genetic defects variably associated with central CH	*PROKR2* (607123)	GPCR	Variable hypopituitarism associated with septo-optic dysplasia or pituitary stalk interruption syndrome	AR, AD
	NFKB2 (164012)	NF	Deficient anterior pituitary with variable immune deficiency (DAVID) syndrome associated with ACTH deficiency and variable GH and TSH defects	AD
	CHD7 (608892)	ATP-dependent helicase	CHARGE syndrome (coloboma, heart anomaly, choanal atresia, retardation, genital and ear anomalies) with ectopic posterior pituitary and variable LH/FSH, TSH, and GH defects	AD
	FGFR1 (136350)	Receptor tyrosine kinase	Kallmann syndrome (KS) and normosmic congenital hypogonadotropic hypogonadism (nCHH), variable association with defects of other pituitary hormones including TSH, septo-optic dysplasia, and ectopic posterior pituitary	AD
	FGF8 (600483)	Growth factor	KS and nCHH, variable associations with defects of other pituitary hormones including TSH, holoprosencephaly, and corpus callosum agenesia	AR
	FOXA2 (600288)	NF	Hypopituitarism with craniofacial and endoderm-derived organ abnormalities and hyperinsulinism	AD

AR autosomal recessive, *AD* autosomal dominant, *NF* nuclear factor, *OMIM* online Mendelian inheritance in men (https://www.ncbi.nlm.nih.gov/omim/), *GPCR* G protein-coupled receptor

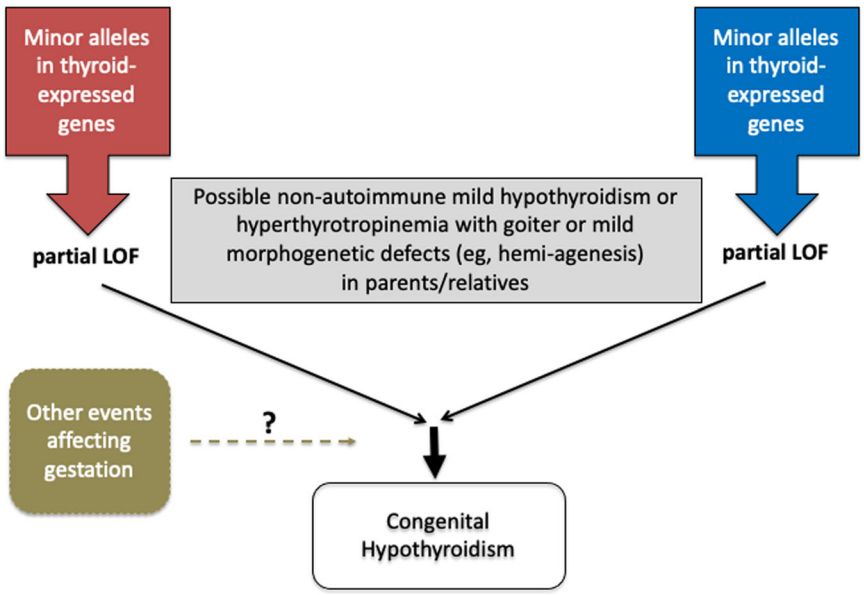

Fig. 1 Proposed model for multifactorial origin of CH. LOF loss of function

within a family or among different individuals. Figure 1 shows a proposed model for multifactorial origin of CH.

In this context, the polymorphic trait of polyalanines of the FOXE1 gene elicited great interest as susceptibility factor. Almost 500 human proteins contain polyalanine stretches, which may act as flexible spacer elements between functional domains [37]. Variations in length of alanine tracts have been shown to cause several human pathological conditions, including congenital malformations and/or mental retardation. Nine disease-associated proteins with alanine tract expansions have been identified and eight of them are transcription factors [38]. Several groups studied the potential relationship between polymorphisms of the FOXE1 polyalanine tract and the thyroid pathogenesis, suggesting a role as a susceptibility factor for TD [39–42]. However, further studies are needed to better understand the actual role of these polymorphic alleles in the pathogenesis of CH.

Alternative mechanisms

The elevated frequency of discordance for CH phenotype between monozygotic (MZ) twins [43] further supports the involvement of alternative or non-Mendelian mechanisms that might predispose to or protect from the full expression of a CH genetic background.

A two-hit mechanism has been proposed as potential alternative mechanism, associating a germline predisposing factor with an additional somatic mutation or epigenetic alteration limited to the thyroid tissue or surrounding structures [44]. However, though somatic mosaicism and monoallelic expression has been reported for some genes associated either with TD and DH [45, 46], somatic mutations were not detected by WES in lymphocyte DNA from MZ twins discordant for TD [47].

Moreover, CGH-array analysis excluded the presence of recurrent copy number variations (CNVs) in CH patients [48].

Epigenetic modifications, and in particular DNA methylation, are emerging as important causes of human disorders. Epigenetics could be also involved in the threefold to fivefold increased risk of CH among children born prematurely or from twin gestations [49]. Indeed, a disturbed gestation, possibly affected by undernutrition, as in the case of premature birth or multiple pregnancies, or a premature adaptation to extra-uterine life might result in an abnormal methylation state, including that of genes involved in thyroid hormone production/metabolism or action [50]. Such an abnormal methylation might lead to a resetting of HPT axis and sustain the increased risk for CH observed in these conditions. To date, systematic methylome analyses have been performed only in the context of thyroid ectopy [51, 52]. However, though ectopy results associated with a different expression pattern, no differences in methylation profile have been found between ectopic and orthotopic thyroid tissues [51] and between peripheral leukocytes of CH cases with ectopy compared to normal controls [52].

Conclusion and perspectives

We provide an update of genetic causes of CH. TD accounts for 65% of CH and a genetic cause is identified in less than 5% of patients in comparison with DH representing 35% of

CH with 50% of identified genetic cause. During the last years, acceleration in genetic elucidation was carried out with the help of new generation sequencing techniques: novel candidate genes in thyroid development, function, and pathways and new thyroid phenotypes in known genes. A total of 17 genes were involved in the pathogenesis of TD, 7 in DH, and 20 in ceCH. The main challenge in the next years will be to enhance our understanding in the pathogenesis of CH by using a new paradigm, the oligogenism model on known genes. Further studies will be needed to establish confirmed diagnosis with oligogenism. Moreover, alternative mechanisms as epigenetic modifications will need to be further explored in order to understand their contribution in CH.

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Compliance with ethical standards

Conflict of interest The authors declare no competing interests.

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