Archives of Pediatrics
Estoppey-Fehlmann J, et al. Arch Pediatr 8: 279
www.doi.org/10.29011/2575-825X.100279
www.gavinpublishers.com

OPEN ACCESS

GAVIN PUBLISHERS

Research Article

Molecular Basis of Childhood-onset Hypothyroidism: Single Centre Screening by Next-Generation Sequencing in a Cohort of Swiss Children

Joëlle Estoppey-Fehlmann[1], Gabor Szinnai[2], Karl Heinimann[3], Britta Seebauer[3], Christa E Flück[1], Marco Janner[1*]

[1]Division of Pediatric Endocrinology, Diabetology and Metabolism, Department of Pediatrics, Inselspital, Bern University Hospital, University of Bern, 3010 Bern Switzerland.

[2]Department of Pediatric Endocrinology, University Children's Hospital Basel, University of Basel, 4056 Basel, Switzerland.

[3]Institute for Medical Genetics and Pathology, University Hospital Basel and University of Basel, 4056 Basel, Switzerland.

[*]**Corresponding author:** Marco Janner, 1Division of Pediatric Endocrinology, Diabetology and Metabolism, Department of Pediatrics, Inselspital, Bern University Hospital, University of Bern, 3010 Bern Switzerland

Citation: Estoppey-Fehlmann J, Szinnai G, Heinimann K, Seebauer B, Flück CE, et al. (2023) Molecular Basis of Childhood-onset Hypothyroidism: Single Centre Screening by Next-Generation Sequencing in a Cohort of Swiss Children. Arch Pediatr 8: 279. DOI: 10.29011/2575-825X.100279

Received Date: 17 July 2023; **Accepted Date:** 25 July 2023; **Published Date:** 29 July 2023.

Abstract

Introduction: The pathogenesis of COH (childhood onset hypothyroidism) has not been fully elucidated but monogenetic as well as oligogenic causes have been shown to play a role. To investigate the molecular cause of COH using next-generation sequencing (NGS) in children with either syndromic hypothyroidism or hypothyroidism with gland in situ. **Methods :** We studied 16 phenotypically well-characterised children with either COH with gland in situ or syndromic COH using an NGS panel of 22 genes, followed by Sanger sequencing confirmation in a population-based, single centre study of a Swiss tertiary care centre for paediatric endocrinology. The pathogenicity of novel variants was assessed by in silico prediction tools. **Results:** In 13 of 16 participants (81%) we found a total of 19 variants (10 pathogenic variants; 62.5%). From these 13 participants only three had been detected at neonatal screening (23%). Of the remaining, five had a TSH level at neonatal screening between 6-12 mU/ml. Variants were most frequently found in *DUOX2* (4) followed by *TG* (3) and *GNAS* (2). In addition, we found variants in *THRB*, *NKX2-1*. One participant showed a ring chromosome 18 and another a large deletion of 18q. We found oligogenic involvement in two participants affecting *ALB, DUOX2, IYD* and *TSHR* and *ALB, TG* and *TSHB,* respectively. One of them is to our knowledge the first case of COH due to biallelic variants in *DUOX2* and *ALB*. In one participant no variant could be found. **Conclusions:** We report on the first single centre screening of COH with NGS in a Swiss tertiary centre. We found 10 pathogenic variants (62.5%). We found oligogenic involvement in two participants. 77% of the participants carrying variants were not diagnosed by neonatal screening.

Citation: Estoppey-Fehlmann J, Szinnai G, Heinimann K, Seebauer B, Flück CE, et al. (2023) Molecular Basis of Childhood-onset Hypothyroidism: Single Centre Screening by Next-Generation Sequencing in a Cohort of Swiss Children. Arch Pediatr 8: 279. DOI: 10.29011/2575-825X.100279

Keywords: Congenital hypothyroidism; Childhood-onset Hypothyroidism; Next-generation sequencing; Oligogenic

Introduction

Congenital hypothyroidism (CH) is one of the most common preventable causes of intellectual disability [1]. In 2020, the incidence of CH in Switzerland was 1:3600 (https://www.neoscreening.ch/wp-content/uploads/2021/12/Screening_JB2020_4_de.pdf, accessed 1.11.2022). CH may be caused by either thyroid dysgenesis (TD), which results from abnormal thyroid gland development and accounts for approximately 85% of cases, or from thyroid dyshormonogenesis (DH) [1-3] TD is mostly sporadic and its clinical phenotype ranges from athyreosis, hypoplasia, and hemiagenesis, to ectopy [1] (Figure 1). However, in approximately 2% of the cases, a genetic cause can be identified [1]. Genes associated with TD are *Paired Box Gene 8* (*PAX8*) [1,4] *NK2 Homeobox 1* (*NKX2-1*) [1,4] *Forkhead Box E1* (*FOXE1*) [1,4] *NK2 Homeobox 5* (*NKX2-5*) [1,5] *TSH-receptor* (*TSHR*) [1,6] *GLI similar 3 (GLIS3)* [7,8] *NETRIN1* [8,9] *JAG1* [8,10] as well as *BOREALIN* [8,11] and *TUBULIN Beta1 Class VI* (*TUBB1*) [8,12]

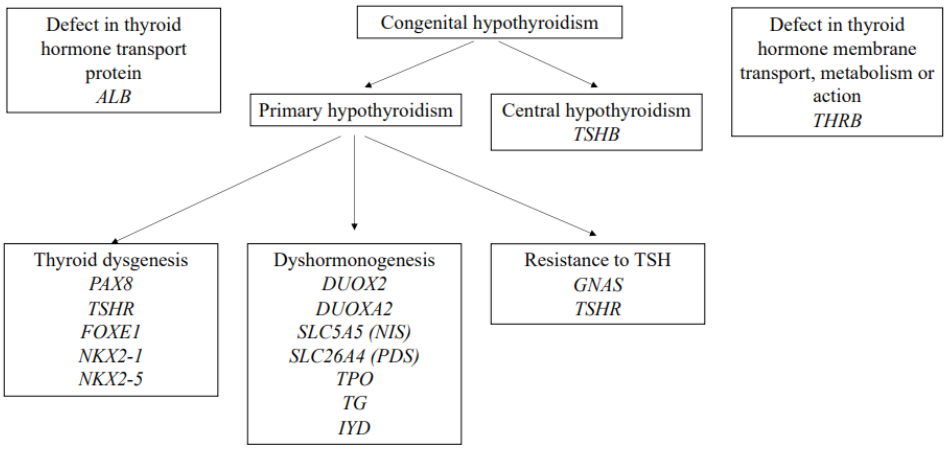

Figure 1: Classification of congenital hypothyroidism; *ALB* albumin, *DUOX2* dual oxidase 2,*DUOXA2* dual oxidase maturation factor 2, *FOXE1* forkhead box E1, *GNAS* G protein α-subunit, *IYD* iodotyrosine deiodinase, *NIS* sodium/iodide symporter, *NKX2-1* NK2 Homebox 1, *NKX2-5* NK2 Homebox 5, *PAX8* paired box gene 8, *PDS* pendrin, *TG* thyroglobulin, *THRB* thyroid hormone receptor β, *TPO* thyroperoxidase, *TSHR* TSH receptor, *TSHB* TSH beta chain

DH is inherited in an autosomal recessive way, mostly due to variants of the genes involved in any of the steps of thyroid hormone biosynthesis [1], like Sodium/iodide symporter *SLC5A5 (NIS)*, Pendrin *SLC26A4 (PDS)*, Dual oxidase 2 *DUOX2*, Dual oxidase 2 maturation factor *DUOXA2*, Thyroperoxidase *TPO*, Thyroglobulin *TG*, Dehalogenase *IYD* and *GNAS* subunit alpha [1,2,8,13-18]. The phenotype of DH is highly variable and shows a gland in situ with or without goitre [2,19].

We defined childhood-onset hypothyroidism (COH) as primary hypothyroidism diagnosed in infancy and childhood after having excluded autoimmune thyroid disease. COH is mainly due to gene variants leading to DH and TD. However, on a biological basis no distinction between CH and COH due to variants in genes responsible for thyroidogenesis or thyroid hormone biosynthesis can be made. Rather it is a question of severity of the phenotype, more severe phenotypes being diagnosed by NBS while less severe phenotypes are diagnosed later in life. Other causes of COH include Down's syndrome and iodine deficiency. In addition, in the last years, the use of next-generation sequencing (NGS) has shown that oligogenic inheritance may play a role in COH [19-21]. Finally, a slight increase of TSH levels can be found as an epiphenomenon of obesity. The detection of genetic causes of CH and COH may improve patient care, particularly in syndromic forms, and provide opportunities for specific genetic counselling to affected families. Therefore, the European Society of Paediatric Endocrinology recommends careful phenotypic characterisation of CH and COH in cases of DH, syndromic hypothyroidism, and in patients with a family history of COH [22].

Our study aimed at investigating the molecular cause of CH and COH in a clinically well-characterised group of children with either syndromic hypothyroidism or hypothyroidism with gland in situ or familial COH using a next-generation sequencing (NGS) panel.

Methods

Participants

105 children who had been followed for primary hypothyroidism between 01.09.2016 – 30.09.2017 at our outpatient clinic for paediatric endocrinology in Bern, Switzerland, were evaluated. All participants underwent clinical examination, had thyroid function tests (TSH, fT4, and fT3), and thyroid sonography. In addition, 36 participants also had I-123 scintigraphy. Scintigraphy was performed in all participants with athyreosis except in one, and all participants with thyroid ectopy except in one, as the ectopic thyroid had already been seen at the ultrasound.

Diagnosis of primary hypothyroidism was done by neonatal screening using capillary whole blood TSH measurements from a heel stick. The threshold value for TSH in a dried blood spot between 4-15 days of life in Switzerland is 15 mU/L. Additionally, in children with hypothyroidism diagnosed later in life thyroid antibodies were measured (anti-TPO, anti-TG) to exclude autoimmune thyroid disease. TSH, fT4, and fT3 were measured by an electrochemiluminescence immunoassay (Roche, Basel, Switzerland) and compared to age-dependent reference ranges.

DNA sequencing

A panel of 22 genes associated with COH (Supplementary Table) was analysed by high throughput sequencing at the Department of Genetics of the University Hospital Basel, Switzerland. Variants were confirmed by Sanger sequencing of single exons. Gene dosage was assessed with the CNV-Analyse-Tool SeqPilot 4.4.0 JSI (Germany). Identified gene variants were searched in the databases HGMD, LVOD, and ClinVar, and their pathogenicity was tested with common in silico predicting tools (SIFT, Variant Taster, PolyPhen2). The classification of the variants as pathogenic, likely pathogenic, variants of uncertain significance, likely benign, and benign was according to the ACMG guidelines [23].

Genetic workup was offered to all participants with COH and gland in situ or with COH and a dysmorphic phenotype, as well as to children with primary hypothyroidism diagnosed later in life with negative thyroid antibodies. In two children with syndromic COH only a karyotype was performed to confirm the clinical diagnosis, one had a ring chromosome 18 and the other a deletion of 18q.

Results

105 children were followed at our clinic between September 2016 and September 2017 for primary hypothyroidism. 41 of them had been diagnosed with COH by neonatal screening (39%), and 64 later in life (61%) Of the participants with a positive neonatal screening, 8 (19.5%) had athyreosis, 19 (46.3%) thyroid ectopy and 14 (34%) gland in situ and were offered genetic testing; 11 families refused genetic testing. 28 (43.8%) of the participants diagnosed later in life had autoimmune thyroid disease and 36 (56.3%) had antibody-negative hypothyroidism. After having excluded hypothyroidism related to Down's syndrome or obesity, the remaining participants (n=24), were labelled to have late-diagnosed hypothyroidism and offered genetic evaluation. Of those, 11 families refused genetic testing. Therefore, finally a total of 16 children (3 with CH diagnosed by neonatal screening and 13 with COH diagnosed later in life) had genetic evaluation. There were no consanguineous families.

Variants

The clinical characteristics and the molecular diagnosis of the participants are summarised in Table 1. We found a total of 19 variants in 13 participants. 10 variants were classified as pathogenic (62.5%). Noteworthy, of these 13 participants, COH had only been detected at neonatal screening in 3 (23%). The other 10 participants had a TSH level at neonatal screening between 6-12 mU/ml. Pathogenic variants in *DUOX2* [4] were most frequent followed by *TG* [3], *GNAS* [2], *THRB*, *TSHR* and *IYD* (one each). We found 6 novel, pathogenic variants in *ALB* [2], *GNAS, NKX2-1, TG* and *TSHB*. In addition, two participants carried a ring chromosome 18 and a large deletion of 18q, respectively (Table 1).

P	G	Age at diagnosis (years)	Gene	Variant (nt)	Variant (AA)	Zygosity	CADD	TSH NS (mU/l)	TSH	fT4	fT3	TSH	fT4	fT3	TG	Imaging	Pheno
1	F	11/12	GNAS	c.308T>C	p.Ile103Thr	het	24.9	7.497				13.95	11.1	6		US	HYPO
2	M	1 1/12	ALB	c.725G>A	p.Arg242His	het	13.7	4.123				7.62	28.7	9.09		US	HYPO
			DUOX2	c.908C>G	p.Pro303Arg	het	26.7										
			IYD	c.323A>G	p.Asn108Ser	het	15.2										
			TSHR	c.1047C>T	p.Asn349=	het	0.9										
3	M	2	TG	c.886C>T	p.Arg296Ter	het	36	2.34				10.95	15.8	6.1	26.7	US	HYPO
4	F	11 7/12	n.d.	n.d.	n.d.							9.85	14	5.8	16.74	US	HYPO
5	M	2	n.d.	n.d.	n.d.			4.734				8.2	14.7	5.76		US	N
6	M	1 1/3	GNAS	c.51_52dup	p.Ala18GlyfsTer41	het	n.a.	7.955				20.05	14.9	7.24		US	HYPO
7	M	7 days	DUOX2	c.602dup	p.Gln202ThrfsTer99	het	n.a.	65.916	>100	7.4	5.8				1981	US, SC	GOITER
			DUOX2	c.2895_2898del	p.Phe966SerfsTer29	het	n.a.										
8	M	11 5/6	DUOX2	c.602dup	p.Gln202ThrfsTer99	het	n.a.	1.041				4.02	14.7	6.38		US	N
9	F	2 11/12	DUOX2	c.602dup	p.Gln202ThrfsTer99	het	n.a.	11.992				3.61	14.5	6.51		US	N
				c2895_2898del	p.Phe966SerfsTer29	het	n.a.										
10	F	1 11/12	ring chromosome 18					6.467				15.18	21.7	6.3		US	HYPO
11	M	10 days	NKX2-1	c.527T>G	p.Leu176Arg	het	29.1	260.079	>100	6	1.92				<0.17	US,SC	ATX
12	M	3/4	18q	terminal deletion				11.818				19.89	13.4	6.45		US	HYPO
13	F	1 1/3	THRB	c.1357C>T	p.Pro453Ser	het	26.7	2.023				1.76	34.6	13.6	21.08	US	N
14	F	11/12	TG	c.1958G>A	p.Gly653Asp	het	24.1	3.196				13.2	15.1	6.1	14.2	US	HYPO
				c.6619G>A	p.Gly2207Ser	het	24.6										
15	F	6 days	ALB	c.382C>A	p.Gln128Lys	het	0.001	20.873	20.3	17.7	8.08				9.66	US,SC	N
			TG	c.6769G>A	p.Ala2257Thr	het	27.1										
			TSHB	c.99G>A	p.Arg33=	het	10.3										
16	F	7 days	no variant					25.671				7.58	12.8	6	10.1	US	HYPO

Table 1: Clinical, biochemical characteristics and molecular genetics of the patients; P: participant, G: gender, M: male, F: female, nt: nucleotide, AA: amino acid. fT4 and fT3 are given in pmol/l and TG (thyroglobulin) in ng/ml, US: ultrasound, SC: scintigraphy, ATX: athyreosis, HYPO: hypoplasia.

Participants 7-9 with *DUOX2* variants were of the same kindred. Participants 7 and 9 were compound heterozygous for the same known variants (c.602dupG; c.2895_2898delGTTC), while their brother (subject 8) was heterozygous for c.602dupG only [24]. Participant 14 was compound heterozygous for two different point-variants in *TG*, one classified as benign variant (c.1958G>A), while the other was a novel variant of unknown significance (c.6619G>A). However, this participant had a slightly elevated TSH level with normal fT4 and fT3 and a hypoplastic gland in situ indicating that the novel variant in *TG* might have an impact on thyroid function. Clinical information on the brother and the sister of participant 3, who showed a known pathogenic variant in *TG,* were both included in this study after consent of the parents although genetic testing had been refused for patient 4 and 5.

We found oligogenic involvement in two participants. Participant 2 showed 4 combined single nucleotide variants in one allele of *ALB, DUOX2, IYD* and *TSHR* each. Only the variant in *DUOX2* was known pathogenic, if biallelic [25]. Variants in the gene coding for albumin (*ALB*) cause Familial Dysalbuminaemic Hyperthyroxinaemia (FDH). FDH is the most common cause of inherited euthyroid hyperthyroxinaemia [26]. Although the novel variant in this participant was of unknown significance, it is noteworthy that the child showed a thyroid hormone pattern with slightly elevated fT3 and fT4 along with an unsuppressed TSH level compatible with FDH. Participant 15 carried monoallelic, single nucleotide variants in *ALB* (variant of unknown significance), *TG* (benign) and *TSHB* (variant of unknown significance). Participant 13 showed a known pathogenic variant in *THRB* and therefore did not have COH.

In participant 16 who had an elevated TSH level at neonatal screening and showed a hypoplastic gland, no variant could be found. Finally, in participant 11 we found a new pathogenic variant in *NKX2-1*: c.527T>G (p.Leu206Arg) that lies in the home box-like domain according to the predicted structure of the protein (NM_001079668.3).

Clinical phenotype

The clinical phenotype of the participants was highly variable, thyroid hypoplasia being the most common morphological finding (Table 1). One of the three participants (participant 7) with *DUOX* variants showed a goitre and had elevated thyroglobulin levels. In contrast to his brother and sister, he had been diagnosed with very high TSH at neonatal screening and received life-long thyroxine replacement treatment. His sister (participant 9) shared the same genotype but did not need replacement therapy although the TSH level at birth was slightly above 10 mU/l. Participants with *GNAS* variants both had hypoplastic gland and required quite high replacement doses of thyroxine. Participants with documented variants in *TG* all had hypoplastic gland. The brother (participant 5) of participant 3, however, had normal gland morphology but needed low dose thyroxine replacement. Only one patient had athyreosis (as shown by both ultrasound and scintigraphy, unmeasurable thyroglobulin very high TSH at 10 days of life); he carried a novel pathogenic variant in *NKX2-1*. He showed the typical clinical phenotype of neonatal respiratory distress, generalised muscle hypotonia and gross motor development delay.

Discussion

In this single centre study, we screened infants with CH showing either a thyroid gland in situ, a dysmorphic phenotype, or primary hypothyroidism diagnosed later in life with negative thyroid antibodies (i.e. childhood onset hypothyroidism: COH) by a NGS panel comprised of 22 genes related to CH and COH.

In two participants with syndromic COH, only a karyotype was performed to confirm the clinical diagnosis of chromosome 18 related disorders because of typical dysmorphic signs. In the remaining 14 participants, a total of 19 variants were detected, and 10 of them were classified as pathogenic according to current ACMG criteria. Three participants carried compound heterozygous variants in the *DUOX2* and *TG* genes. Two participants showed monoallelic, oligogenic variants in *ALB, DUOX2, IYD* and *TSHR* and *ALB, TG, TSHB* genes, respectively.

One participant with a well-documented thyroid agenesis carried a novel pathogenic variant of *NKX2-1:* c.527T>G (p.Leu206Arg), that lies in the homeobox-like domain. To our best knowledge, this is the first case in the literature of a NKX2-1 variant leading to athyreosis.

Interestingly, only three participants had been diagnosed with congenital hypothyroidism by neonatal screening. This low number, however, is most likely biased by the small number of the participants in the participants diagnosed by neonatal screening, due to the high refusal rate of their guardians. All participants diagnosed later in life (COH) had thyroid gland in situ.

Variants were most frequently found in *DUOX2* (n=4) followed by *TG* (n=3) and *GNAS* (n=2). Due to the small number of the study cohort, a comparison to prevalence data from the literature makes little sense. The siblings of participant 3 who was a carrier of a heterozygous, pathogenic variant in *TG* (c.886C>T), both showed slightly elevated TSH (8-11 mU/L) at diagnosis and eventually needed thyroxine replacement treatment. In addition, the sister had a hypoplastic gland suggesting that both children, in whom genetic analysis had been refused, might share the same known gene variant. Several studies have described that monoallelic carriers of this variant show a variable phenotype of COH like our patient [21,27-29].

Alternatively, oligogenic involvement has been discussed as an explanation for intrafamilial variability in COH [30]. In our study two participants (2 and 15) showed oligogenic involvement. Participant 2 showed a known pathogenic variant in *DUOX2* [25] and a new variant in *ALB* (c.7725G>A) of unknown significance. Variants in *ALB* result in familial dysalbuminaemic hypothyroxinaemia (FDH). FDH is the most common cause of inherited euthyroid hypothyroxinaemia [26] FDH is characterised by either elevated fT4 and fT3 or elevated fT4 with normal fT3 in presence of unsuppressed TSH with or without goiter [26] Interestingly, participant 2 had thyroid hormone levels slightly above the upper limit of the reference range in the presence of unsuppressed TSH. This is an unexpected finding if we only consider the pathogenic *DUOX2* variant. A possible explanation could be that this particular oligogenic involvement causes an intermediate phenotype between *DUOX2* deficiency and findings compatible with a minor form of FDH. To our best knowledge, this is the first case of a biallelic variant affecting *DUOX2* and *ALB*.

Participant 15 had a normal-sized gland and showed a variant of unknown significance in *TG* and a new variant in *ALB* as well as a new variant in *TSHB*, both of unknown significance. However, this girl was detected by newborn screening as having compensated CH and needed thyroxine replacement treatment at the usual dosage. Her thyroglobulin level was low normal and she originated from a region of adequate iodine intake. Therefore, this phenotype could be explained either by a pathogenic variant in *TG* [18] or by the presence of an undetected second hit.

In two siblings (7 and 9) we found two different variants in *DUOX2*; their brother, participant 8, shared only the c602dupG variant with them. While participants 8 and 9 did not show any thyroid phenotype, participant 7 had a goitre and had been diagnosed at birth with elevated TSH, although he needed only transitory thyroxine replacement treatment. This high interfamilial variability is in line with the current knowledge about *DUOX2* variants [31,32].

Participant 14 showed two different variants in TG. One of them (c.1958G>A) is likely benign, and the second (c.6619G>A) had not been described previously. The novel variant tested pathogenic by different prediction tools and could therefore be an explanation for the phenotype of the child. Alternatively, additional undetected variants or other contributing pathophysiological factors might be considered [32] GNAS variants leading to pseudo hypoparathyroidism 1a associated with TSH resistance are rare causes of COH [33]. We found two variants in GNAS in participants showing a typical phenotype of pseudohypoparathyroidism with Albright's osteodystrophy. One participant carried a new variant, c.51_52dup p.GGhet.

This study has several limitations: Although the assessment by using a NGS panel was done prospectively, the collection of former laboratory data was done retrospectively. This explains why the clinical data are partly incomplete. Second, the sample size is small. Therefore, no statements can be done on the prevalence of the different variants in our population. Third, the parents of the participants were not studied. And finally, no functional studies have been performed to further investigate the functional consequences of the six newly identified variants.

In conclusion, we provide the first population-based genetic data of COH in a small, single centre, Swiss cohort. We found 10 pathogenic variants (62.5%). NGS panel diagnostics allowed us to find oligogenic involvement in two participants. One of them is probably the first case of COH due to biallelic variants in *DUOX2* and *ALB*. In addition, we describe the first variant of NKX2-1 leading to thyroid agenesis. 77% of the participants carrying variants were not diagnosed by neonatal screening. This

study also confirms that the genetics of COH is complex, and that alternative approaches in basic research are needed to better understand thyroid embryogenesis and to unravel the complex role of genes, transcription factors and maturation factors in thyroid development.

Acknowledgement

We thank E. Albertin (Neonatal Screening Switzerland) and Dr. A. Cremonesi (Neonatal Screening Switzerland) for the results of neonatal screening of the patients with genetic variants and ESPE for the financial support.

Statement of ethics

All investigations were part of an ethically approved protocol and the research was conducted ethically in accordance with the World Medical Association Declaration of Helsinki. All patients gave general consent to the anonymized use of clinical data in their files. For genetical analysis written informed consent was obtained from the participants' parent/legal guardian/next of kin to participate in the study and for publication of the details of their medical case. For the genetical analysis the study protocol was reviewed and approved by Ethikkommission Nordwest- und Zentralschweiz, project number 2018-01770

Declarations of interest

The authors have nothing to disclose.

Funding

This project was supported by a European Society for Paediatric Endocrinology (ESPE) Research Unit Grant to GS.

Contributions of the authors

MJ conceived, designed, and drafted the work. Data collection and analysis were performed by JEF. MJ and CEF revised it for important intellectual content and gave the final approval of the version to be published. GS, BS and KH did all the molecular genetics. All the authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Data availability

The data of this study are not openly available. All data generated or analysed during this study are included in this article. Further enquiries can be directed to the corresponding author.

References

1. Szinnai G (2014) Clinical Genetics of Congenital Hypothyroidism. Endocrin Dev 26: 60-78.
2. Persani L, Rurale G, Filippis T de, Galazzi E, Muzza M, Fugazzola L (2018) Genetics and management of congenital hypothyroidism. Best Pract Res Clin Endocrinol Metab 32: 387-396.
3. Peters C, Trotsenburg ASP van, Schoenmakers N (2018) DIAGNOSIS OF ENDOCRINE DISEASE: Congenital hypothyroidism: update and perspectives. Eur J Endocrinol 179: R297-317.
4. Fernández LP, López-Márquez A, Santisteban P (2015) Thyroid transcription factors in development, differentiation and disease. Nat Rev Endocrinol 11: 29-42.
5. Dentice M, Cordeddu V, Rosica A, Ferrara AM, Santarpia L, et al. (2006) Missense Mutation in the Transcription Factor NKX2–5: A Novel Molecular Event in the Pathogenesis of Thyroid Dysgenesis. J Clin Endocrinol Metab 91: 1428–1433.
6. Persani L, Calebiro D, Cordella D, Weber G, Gelmini G, et al. (2010) Genetics and phenomics of hypothyroidism due to TSH resistance. Mol Cell Endocrinol 322: 72-82.
7. Dimitri P, Warner JT, Minton JAL, Patch AM, Ellard S, et al. (2011) Novel GLIS3 mutations demonstrate an extended multisystem phenotype. Eur J Endocrinol 164: 437-443.
8. Stoupa A, Kariyawasam D, Muzza M, Filippis T de, Fugazzola L, et al. (2021) New genetics in congenital hypothyroidism. Endocrine 71: 696-705.
9. Opitz R, Hitz MP, Vandernoot I, Trubiroha A, Abu-Khudir R, et al. (2015) Functional Zebrafish Studies Based on Human Genotyping Point to Netrin-1 as a Link Between Aberrant Cardiovascular Development and Thyroid Dysgenesis. Endocrinology 156: 377-388.
10. Filippis T de, Marelli F, Nebbia G, Porazzi P, Corbetta S, et al. (2016) JAG1 Loss-Of-Function Variations as a Novel Predisposing Event in the Pathogenesis of Congenital Thyroid Defects. J Clin Endocrinol Metab 101: 861-870.
11. Carré A, Stoupa A, Kariyawasam D, Gueriouz M, Ramond C, et al. (2017) Mutations in BOREALIN cause thyroid dysgenesis. Hum Mol Genet 599-610.
12. Stoupa A, Adam F, Kariyawasam D, Strassel C, Gawade S, et al. (2018) TUBB1 mutations cause thyroid dysgenesis associated with abnormal platelet physiology. Embo Mol Med 10: e9569.
13. Lemos MC, Thakker RV (2015) GNAS Mutations in Pseudohypoparathyroidism Type 1a and Related Disorders. Hum Mutat 36:11-19.
14. Spitzweg C, Morris JC (2010) Genetics and phenomics of hypothyroidism and goiter due to NIS mutations. Mol Cell Endocrinol 322: 56-63.
15. Bikker H, Vulsma T, Baas F, Vijlder JJM de (1995) Identification of five novel inactivating mutations in the human thyroid peroxidase gene by denaturing gradient gel electrophoresis. Hum Mutat 6: 9-16.
16. Citterio CE, Targovnik HM, Arvan P (2019) The role of thyroglobulin in thyroid hormonogenesis. Nat Rev Endocrinol 15 :323-338.
17. Afink G, Kulik W, Overmars H, Randamie J de, Veenboer T, et al. (2008) Molecular Characterization of Iodotyrosine Dehalogenase Deficiency in Patients with Hypothyroidism. J Clin Endocrinol Metab 93: 4894–4901.
18. Citterio CE, Rivolta CM, Targovnik HM (2021) Structure and genetic variants of thyroglobulin: Pathophysiological implications. Mol Cell Endocrinol 528: 111227.
19. Stoupa A, Chehade GAH, Chaabane R, Kariyawasam D, Szinnai G, et al. (2021) High Diagnostic Yield of Targeted Next-Generation Sequencing in a Cohort of Patients With Congenital Hypothyroidism Due to Dyshormonogenesis. Front Endocrinol 11: 545339.

20. Filippis T de, Gelmini G, Paraboschi E, Vigone MC, Frenna MD, et al. (2017) A frequent oligogenic involvement in congenital hypothyroidism. Hum Mol Genet 26: 2507-2514.

21. Oliver-Petit I, Edouard T, Jacques V, Bournez M, Cartault A, et al. (2021) Next-Generation Sequencing Analysis Reveals Frequent Familial Origin and Oligogenism in Congenital Hypothyroidism With Dyshormonogenesis. Front Endocrinol 12: 657913.

22. Léger J, Olivieri A, Donaldson M, Torresani T, Krude H, et al. (2014) European Society for Paediatric Endocrinology Consensus Guidelines on Screening, Diagnosis, and Management of Congenital Hypothyroidism. Horm Res Paediat 81: 80-103.

23. Richards S, Aziz N, Bale S, Bick D, Das S, et al. (2015) Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med 17: 405-424.

24. Varela V, Rivolta CM, Esperante SA, Gruñeiro-Papendieck L, Chiesa A, et al. (2006) Three Mutations (p.Q36H, p.G418fsX482, and g.IVS19-2A>C) in the Dual Oxidase 2 Gene Responsible for Congenital Goiter and Iodide Organification Defect. Clin Chem 52: 182-191.

25. Löf C, Patyra K, Kuulasmaa T, Vangipurapu J, Undeutsch H, et al. (2016) Detection of Novel Gene Variants Associated with Congenital Hypothyroidism in a Finnish Patient Cohort. Thyroid 26: 1215-1224.

26. Dieu X, Bouzamondo N, Briet C, Illouz F, Moal V, et al. (2020) Familial Dysalbuminemic Hyperthyroxinemia: An Underdiagnosed Entity. J Clin Med 9: 2105.

27. Zdraveska N, Kocova M, Nicholas AK, Anastasovska V, Schoenmakers N (2020) Genetics of Gland-in-situ or Hypoplastic Congenital Hypothyroidism in Macedonia. Front Endocrinol 11: 413.

28. Tanaka T, Aoyama K, Suzuki A, Saitoh S, Mizuno H (2020) Clinical and genetic investigation of 136 Japanese patients with congenital hypothyroidism. J Pediatric Endocrinol Metab 33: 691-701.

29. Wang H, Kong X, Pei Y, Cui X, Zhu Y, et al. (2020) Mutation spectrum analysis of 29 causative genes in 43 Chinese patients with congenital hypothyroidism. Mol Med Rep 22: 297-309.

30. Muzza M, Rabbiosi S, Vigone MC, Zamproni I, Cirello V, et al. (2014) The Clinical and Molecular Characterization of Patients With Dyshormonogenic Congenital Hypothyroidism Reveals Specific Diagnostic Clues for DUOX2 Defects. J Clin Endocrinol Metabolism. 99: E544-553.

31. Dufort G, Larrivée-Vanier S, Eugène D, Deken XD, Seebauer B, et al. (2019) Wide Spectrum of DUOX2 Deficiency: From Life-Threatening Compressive Goiter in Infancy to Lifelong Euthyroidism. Thyroid 29: 1018-1022.

32. Deken XD, Miot F (2019) NADPH Oxidases, Methods and Protocols. Methods Mol Biology 1982: 667-693.

33. Mantovani G, Bastepe M, Monk D, Sanctis L de, Thiele S, et al. (2020) Recommendations for Diagnosis and Treatment of Pseudohypoparathyroidism and Related Disorders: An Updated Practical Tool for Physicians and Patients. Horm Res Paediatr 93: 182-196.

Category		Gene	Inheritance Mode	Disease Association	OMIM
GENETIC FORMS OF HYPOTHYROIDISM	Central Hypothyroidism: Pituitary or hypothalamic	IGSF1	XLD	Central hypothyroidism and testicular enlargement	300888
		TRHR	AR	Thyrotropin‐releasing hormone resistance, generalized	188545
		TSHB	AR	Hypothyroidism, congenital, non goitrous 4	275100
	Thyroidal Hypothyroidism: Dysgenesis	PAX8	AD	Hypothyroidism, congenital, due to thyroid dysgenesis or hypoplasia	218700
		FOXE1	AR (AD)	Bamforth‐Lazarus syndrome (Hypothyroidism, thyroidal, or athyroidal)	241850
		NKX2‐1	AD	Choreoathetosis, hypothyroidism, and neonatal respiratory distress	610978
		NKX2‐5	AD	Hypothyroidism, congenital non goitrous, 5	225250
	Thyroidal Hypothyroidism: Dyshormonogenesis	SLC5A5, NIS	AR	Thyroid dyshormonogenesis 1	274400
		TPO	AR	Thyroid dyshormonogenesis 2A	274500
		TG	AR	Thyroid dyshormonogenesis 3	274700
		IYD, DEHAL1	AR	Thyroid dyshormonogenesis 4	274800
		DUOXA2	AR	Thyroid dyshormonogenesis 5	274900
		DUOX2	AR	Thyroid dyshormonogenesis 6	607200
		SLC26A4, PDS	AR	Pendred syndrome	274600
	Thyroidal Hypothyroidism: Resistance to TSH or GNAS	TSHR	AR	Hypothyroidism, congenital, non goitrous 1	275200
			AD	Hyperthyroidism, nonautoimmune	609152
		GNAS	AD	(Pseudo) Pseudohypoparathyroidism; progressive osseous heteroplasia	103580

DISORDERS OF THYROID HORMONE TRANSPORT PROTEINS	ALB	AD	Familial dysalbuminemic hyperthyroxinemia	615999
	SERPINA7, TBG	XLD	Thyroxine - binding globulin quantitative trait locus	300932
DISORDERS OF THYROID HORMONE MEMBRANE TRANSPORT, METABOLISM, OR ACTION	SLC16A2, MCT8	XLD	Allan - Herndon - Dudley syndrome	300523
	SECISBP2	(AR)	Thyroid hormone metabolism, abnormal	609698
	THRA	AD	Hypothyroidism, congenital, non goitrous, 6	614450
	THRB	AD/AR	Thyroid hormone resistance	188570
GENETIC FORMS OF HYPERTHYROIDISM	TSHR	AD/AR	Hyperthyroidism, non - autoimmune, sporadic, or familial	603373
	GNAS		McCune - Albright syndrome, somatic, mosaic	174800

Supplementary Table: Gene panel for congenital disorders of the thyroid; We used a gene panel of the Labor Molekulargenetik, Institut für Medizinische Genetik und Pathologie, Universistätsspital Basel, 4031 Basel, Schweiz; AD autosomal - dominant, AR autosomal - recessive, XLD X - linked, IGSF1 immune globulin superfamily member 1, ALB albumin, DEHAL1 dehalogenase 1, IYD iodotyrosine deiodinase, DUOX2 dual oxidase 2. DUOXA2 dual oxidase maturation factor 2, FOXE1 forkhead box E1, GNAS G protein α-subunit, NIS (SLC5A5) sodium/iodide symporter, NKX2-1 NK2 Home box 1, NKX2-5 NK2 Home box 5, PAX8 paired box gene 8, PDS (SLC26A4) pendrin, TG thyroglobulin, THRA thyroid hormone receptor, THRB thyroid hormone receptor β, TPO Thyroperoxidase, TRHR Thyrotropin - releasing hormone, TSHB TSH beta chain, TSHR TSH receptor, MCT8 monocarboxylic transporter 8, SECISBP2 Selenocysteine Insertion Sequence-Binding Protein 2