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ORIGINAL ARTICLE

Clinical, biochemical, and molecular findings in Argentinean patients with goitrous congenital hypothyroidism

Ana Chiesa · Carina M. Rivolta · Héctor M. Targovnik · Laura Gruñeiro-Papendieck

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Abstract We describe the clinical, biochemical, and molecular findings of a cohort of Argentinean patients with congenital hypothyroidism (CH) and goiter studied to characterize iodide organification and thyroglobulin (TG) defects. 20 CH patients (16 unrelated) were grouped according to serum TG levels and a perchlorate discharge test (PDT) in: group 1 (G1): nine patients with high TG and PDT $> 10\%$ who were studied for tiroperoxidase (TPO), dual oxidase 2 (DUOX2), and dual oxidase A2 (DUOXA2) defects and group 2 (G2): 11 patients with low TG and PDT $< 10\%$ studied for TG defects. Goiter characteristics, outcome, and TT_4 and TT_3 levels without treatment were compared between groups. 6/9 G1 patients harbored mutations in TPO gene and 3/9 in DUOX2 gene. In G2, mutations of TG gene were found in 3/11 homozygous, 5/11 compound heterozygous, and 3/11 heterozygous patients. Goiter was only evidenced by thyroid scan in the neonatal period in both groups; was moderately enlarged in patients diagnosed during infancy. In the late detected patients, goiter was big and nodular in G1 while diffuse and moderate in G2. Early detected patients grew and developed normally while those diagnosed late were severely mentally retarded in G1 and only mildly retarded in G2. Thyroid hormone levels of G1 were significantly lower than those of G2 $P < 0.01$. Molecular approach to characterize defects in organification and TG defects was optimized by TG measurements and PDT. Clinical and biochemical differences based on molecular findings will allow further investigations on genotype–phenotype relationships.

Keywords Thyroperoxidase gene · DUOX2 gene · Thyroglobulin gene · Mutation · Iodide organification defect · Hypothyroidism · Congenital goiter

Abbreviations

CH	Congenital hypothyroidism
TG	Thyroglobulin
TPO	Thyroid peroxidase
DUOX2	Dual oxidase 2
DUOXA2	Dual oxidase A2
(DEHAL1)	Iodotyrosine dehalogenase 1
PDT	Perchlorate discharge test
TSH	Thyrotropin
TT_4	Total thyroxin
TT_3	Total triiodotironine

Introduction

Congenital hypothyroidism (CH) is the most common innate endocrine disease. Detection of CH by neonatal screening has been a major achievement because early diagnosis and treatment result in normal development in nearly all cases. An additional benefit of neonatal screening has been the elucidation of the incidence of CH as well as the prevalence of its various causes. CH occurs in 1/1900–1/3000 neonates and is one of the most preventable

A. Chiesa (✉) · L. Gruñeiro-Papendieck
División Endocrinología, Hospital de Niños "Ricardo Gutiérrez", Centro de Investigaciones Endocrinológicas, CEDIE-CONICET, Gallo 1330, 1425 Buenos Aires, Argentina
e-mail: achiesa@cedie.org.ar

C. M. Rivolta · H. M. Targovnik
Laboratorio de Biología Molecular, Cátedra de Genética y Biología Molecular, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, 1113 Buenos Aires, Argentina

causes of mental retardation through the world; CH is in fact four to five times more common than Phenylketonuria for which newborn screening was initially developed [1, 2].

Whereas the majority of the cases of CH are due to dysembryogenesis or dysgenesis of the thyroid gland (agenesis, ectopia, and hypoplasia), one-fifth of these hypothyroid newborns have a defect in thyroid hormonogenesis (dyshormonogenesis), which may lead to goitrous hypothyroidism. Finally, in less than 10% of individuals diagnosed with CH the abnormality is found to be transient [1, 2].

The reference to inherited goiter and CH was suggested 100 years ago [3, 4]. However, since the pioneer studies of Stanburry et al. in 1950 [5] reporting siblings with iodide organification defect, the understanding of newborn errors of thyroid synthesis grew enormously.

Various genetic defects impairing biosynthesis of thyroid hormone have been identified in patients with congenital goiter. Thyroid insufficiency may be caused by defects of iodide organification due to defects of the thyroperoxidase (TPO) [6–18], dual oxidase 2 (DUOX2) [19–23], dual oxidase A2 (DUOXA2) [24], and pendrin [25]; otherwise mutations in the TG gene originate a defective TG [26–44], a large glycoprotein secreted into the follicular lumen that serves as the matrix for synthesis of T_4 and T_3 and their storage. Patients with iodotyrosine dehalogenase deficiency may develop goiter with hypothyroidism, when dietary iodide is limited. Moreover, recently, the first mutations in the iodotyrosine dehalogenase 1 (DEHAL1) gene have also been reported [45, 46].

Molecular technology is now able to identify the genes responsible for the thyroid defects and more trouble free methods can be performed allowing a definitive diagnosis in the affected babies.

The information provided by the molecular approach will hopefully help to explain the difference in this patients' outcome, to develop new diagnostic and therapeutic strategies and to ensure adequate genetic counseling as well as optimal care and support for these patients.

Nevertheless, molecular studies may be expensive and could be optimized when patients are carefully selected taking into account the efficiency of the diagnostic tools and the prevalence of the mutations searched.

Here, we report the clinical, biochemical, and molecular findings in a cohort of patients referred to us in the last 2 decades with CH and goiter who underwent studies for iodide organification and TG defects.

Patients and methods

We studied 20 patients from 16 unrelated families with CH and goiter, 10 of them detected by newborn screening, all without thyroid autoimmunity (negative anti-TPO and anti-TG antibodies) and treated with L-thyroxin at recommended doses since the initial diagnosis [47]. All patients were from areas with adequate iodide supply. They consulted over a period of 20 years and were followed in the Endocrine Division of the Buenos Aires Children's Hospital for a mean period of 12 years (range 4–20 years).

High TSH levels confirmed the diagnosis of hypothyroidism and before starting treatment all patients had a thyroid ultrasound or ^{99}Tc scan showing a normally located and variably enlarged thyroid gland.

At the time of re-evaluation, the treatment was interrupted for a month, and thyroid function was assessed and a potassium perchlorate discharge test (PDT) performed as described below.

Serum TSH, total thyroxin (TT_4), and total triiodotironine (TT_3) were determined by electrochemiluminiscence, (Elecsys, Roche Diagnostic Corporation, Indianapolis, IN USA). Serum thyroglobulin (TG) was measured by Delphia IFMA, (Perkin Elmer Wallac Turku Finland). Anti-TPO and anti-TG antibodies were determined by CLIA (Immulite DPC Diagnostic Products Corporation. Los Angeles CA USA).

The PDT patients were given 25 μCi of radioiodine orally and thyroidal uptake of the isotope was monitored every 15 min for an hour. At this point, 500 mg of potassium perchlorate was administered orally and sequential uptakes were measured at 15 min intervals for an additional 2 h. The discharge was calculated as the difference between uptake at the time of potassium perchlorate administration and the uptake 2 h after. Values above 10% were considered as a failure to retain the administered radioiodine. This is usually due to an abnormal iodide organification.

Afterward, patients were divided according to TG levels and/or potassium perchlorate discharge test in two groups

Group 1

Table 1 included nine patients from eight unrelated families with high TG levels and potassium perchlorate discharge test >10% who were assigned to the molecular study for organification defects. In them, the TPO and DUOX2 genes were studied by SSCP analysis of PCR-amplified genomic DNA. Samples showing an aberrant SSCP pattern were directly sequenced with the Taq polymerase-based chain terminator method (fmol, Promega, Madison, WI) [13, 21]. In the patients in whom only one allele carrying of TPO gene mutation was found, all exons and intron/exon boundaries of the TPO and DUOXA2 genes were sequenced using ABI Prism Big Dyedeoxyterminator cycle sequencing Kit (Applied Biosystems, Weiterstadt—Germany) on the ABI Prism 3100 DNA sequencer (Applied Biosystems) [17].

Table 1 Clinical, biochemical, and molecular findings of patients with iodide organification defect

Patients	Age at diagnosis/ re-evaluation	TSH (mU/l)	PDT (%)	TG (ng/dl)	TT4 (μg/dl)	TT3 (ng/dl)	Thyroid gland	Neurologic outcome	Gene	Exon	Nucleotide change	Consequence at protein level
1	10 days/2.5 years	70	80	1016	1	ND	Enlarged in ^{99}Tc scan	Normal	TPO	5	c.387delC	p.N129fsX208
										8	c.1159G>A	p.G387R
2	9 days/6.5 years	200	77	314	1.6	19.5	Enlarged in ^{99}Tc scan	Normal	TPO	4	c.215delA	p.Q72fsX86
										14	c.2422T>C	p.C808R
3	3 years/12 years	>100	69	1683	1	105	Big nodular goiter	Severe retardation	TPO	8	c.920A>C	p.N307T
										NI	NI	NI
4	11.8 years/16.7 years	160	99	3660	0.20	19.5	Big nodular goiter	Severe retardation	TPO	9	c.1496C>T	p.P499L
										NI	NI	NI
5	4 months/11.5 years	70	84	ND	0.5	44	Mild diffuse	Severe retardation	TPO	8	c.1186–1187insGGCC	p.R396fsX472
										NI	NI	NI
6	11.8 years/12.9 years	375	80	4012	0.4	52	Big nodular goiter	Severe retardation	TPO	8	c.920A>C	p.N307T
										NI	NI	NI
7	3 days/5 years	156	46	431	2.9	126	Enlarged in ^{99}Tc scan	Normal	DUOX2	2	c.1086G>C	p.Q36H
										21	c.2895–2898delGTTC	p.S965fsX994
8[a]	8 months/4.5 years	>100	68	479	0.8	ND	Mild diffuse	Mild retardation	DUOX2	11	c.1253delG	p.G418fsX482
										Intron19	g.IVS19-2A>C	Skipping exon 20
9[a]	1 month/4 years	>100	60	1314	<1	36	Enlarged in ^{99}Tc scan	Normal	DUOX2	11	c.1253delG	p.G418fsX482
										Intron19	g.IVS19-2A>C	Skipping exon 20
Reference values		0.5–5	<10	<1 year: 11–98 >1 year: 2–30	6–14	80–200						

The values reflect the hormonal levels before levothyroxine substitution or after 1 month LT4 withdrawal. *ND* not determined; *NI* not identified. [a] brothers

Patients 7, 8, and 9 named as II-2 (Family 1), II-2 (Family 2), and II-4 (Family 2), respectively, in (21)

Conversion to SI units TT_4: μg/dl × 12.87 = nmol/l, TT_3: ng/dl × 0.01536 = nmol/l, TG: ng/ml × 1.515 = pmol/l

Group 2

Table 2 included 11 patients from eight unrelated families that had low TG levels, and PDT < 10% who underwent molecular studies for TG disorders. In this group, the complete or partial coding regions of the human TG gene, along with the flanking regions of each intron from patients 10–13 to 16–19 were sequenced directly on an ABI Prism 3100 analyzer (Applied Biosystems) [37, 38, 42]. Patients 14, 15, and 20 were studied by SSCP analysis and exons with aberrant SSCP patterns were sequenced [42].

Sequence variants were numbered according to TPO, DUOX2, and TG mRNAs reference sequences reported in GenBank (TPO: NM 000547, DUOX2: NM 014080, TG: NM 003235). The A of the ATG of the initiator methionine codon was denoted as nucleotide +1. The codon for the initiator methionine was codon 1.

Student's *t* test was used to compare TT_4 and TT_3 levels at diagnosis or re-evaluation between groups.

Mental development of each patient was assessed in the last clinical visit and scored with the information provided by parents, teachers, or caregivers and the subjective judgment of the physician as: (1) normal: patients with normal skills and academic performance for age, (2) mild retardation patients with mild delay and poor academic achievement, and (3) severe retardation: patients with deficient development requiring special rehabilitation.

Written informed consent was obtained from the parents and patients involved in this study. The research project was approved by the Ethical Committee of the Hospital Ricardo Gutierrez from Buenos Aires and Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires.

Results

The clinical, biochemical, and molecular data of patients of group 1 with suspected diagnosis of iodide organification defect are shown in Table 1.

Four out of nine patients (1, 2, 7, and 9) were detected and treated in the neonatal period as the result of confirmation of an abnormal neonatal screening. Two children (5 and 8) were diagnosed in the first year of life (4 and 8 months) and already presented with clinical suspicion of hypothyroidism. Finally, the remaining three patients of group 1 (3, 4, and 6) were detected between 3 and 11.8 years of age and all consulted for goiter and profound signs and symptoms of hypothyroidism.

Goiter before starting treatment in this group was clinically unnoticed in the neonatal period and only evidenced as an enlarged thyroid in the ^{99}Tc thyroid scan, moderate and evidenced as an enlarged diffuse palpable thyroid in infancy, and very big (between 50 and 60 ml) with nodules in the three patients detected late in childhood. Patients in this group had high TSH and TG serum levels with very low serum TT_4 and low TT_3 levels. Growth and development were normal in the four early detected patients. All late detected patients were mentally retarded.

The investigation of TPO, DUOX2, and DUOXA2 genes found that six patients in group 1 harbored mutations in the TPO gene. Two were compound heterozygous for TPO mutations: the first had the p.N129fsX208/p.G387R mutations (patient 1). While the c.387delC mutation (p.N129fsX208) generates a truncate protein of 207 amino acids which lacks of TPO activity, the c1159G>A mutation (p.G387R) is located in the animal hem peroxidase domain and would seriously interfere with TPO activity due to the fact that glycine is a neutral non-polar amino acid while arginine is a basic one [13, 17].

The second patient was a heterozygous p.C808R/p.Q72fsX86 (patient 2). The c.215delA mutation in exon 4 eliminates all functional domains of the TPO generating a truncated protein of 85 amino acids (p.Q72fsX86). The other allele was affected by the c.2422T>C mutation in exon 14 that causes a missense substitution p.C808R located in the calcium binding epidermal growth factor (EGF) like domain of TPO. The substitution of cysteine by arginine disables the formation of disulfide bonds and probably disrupts the tertiary structure of the EGF-like domain, resulting in inactivated TPO [13, 17].

Only one TPO mutation was identified in patients 3 to 6. All of them were simply heterozygous for p.N307T, p.P499L, p.R396fsX472, or p.N307T [13, 17].

The study for DUOX2 gene completed the study of this group and allowed to characterize other three patients from two unrelated families (7 to 9) that were compound heterozygous carrying four different mutations. Patient 7 harbored p.Q36H/p.S965fsX994 mutations, a missense mutation and a deletion which produced a frameshift with a putative premature stop codon. While the first one, affects the TPO like domain causing a structural instability that leads to deficient DUOX2 function the other gives origin to a truncated protein without activity [21].

Patients 8 and 9 were brothers and compound heterozygous for p.G418fsX482/skipping of exon 20. The first mutation (c.1253delG) is a deletion that leads to a premature stop codon generating a truncated protein and the second an adenine to cytosine transversion at position-2 of the splice acceptor site in intron 19 with skipping of exon 20 of the DUOX2 gene leading to the possible elimination of exon 20 of the DUOX 2 gene with a frameshift and a premature stop in exon 21 [21].

Table 2 shows the clinical, biochemical, and molecular data of patients of group 2 with suspected diagnosis of TG defect.

Table 2 Clinical, biochemical, and molecular findings of patients with thyroglobulin defect

Patients	Age at diagnosis/ re-evaluation	TSH (mU/l)	PDT (%)	TG (ng/dl)	TT4 (μg/dl)	TT3 (ng/dl)	Thyroid gland	Neurologic outcome	Exon	Nucleotide change	Consequence at protein level
10	3 days/NR	>100	ND	0.9	3.3	133	Enlarged in ^{99}Tc scan	Normal	7	c.886 C>T	p.R277X
									7	c.886 C>T	p.R277X
11	1.3 years/NR	>50	0	0.9	1.5	115	Diffuse goiter	Mild retardation	7	c.886 C>T	p.R277X
									NI	NI	NI
12	4 years/NR	74.7	0	3.3	4.1	196	Big irregular goiter	Mild retardation	7	c.886 C>T	p.R277X
									38	c.6701 C>A	p.A2215D
13	3 days/3 years	60	0	0.9	2.7	175	Enlarged in ^{99}Tc scan	Normal	5	c.548 G>A	p.C164Y
									7	c.759–760insA	p.L234fsX237
14[c]	15 days/4 years	>100	0	1.4	2.8	108	Enlarged in ^{99}Tc scan	Normal	38	c.6701 C>A	p.A2215D
									38	c.6701 C>A	p.A2215D
15[c]	15 days/NR	>100	ND	6.9	1.6	87	Enlarged in ^{99}Tc scan	Normal	38	c.6701 C>A	p.A2215D
									38	c.6701 C>A	p.A2215D
16[b]	3.1 years/NR	140	0	0.9	4.2	143	Diffuse goiter	Normal	7	c.886 C>T	p.R277X
									NI	NI	NI
17[b]	2 years//NR	20	0	ND	3	ND	Diffuse goiter	Normal	7	c.886 C>T	p.R277X
									NI	NI	NI
18[a]	3 days/5 years	>50	0	ND	3.7	182	Enlarged in ^{99}Tc scan	Normal	7	c.886 C>T	p.R277X
									22	c.4588 C>T	p.R1511X
19[a]	3 years/NR	>60	ND	0.9	4.7	195	Diffuse goiter	Normal	7	c.886 C>T	p.R277X
									22	c.4588 C>T	p.R1511X
20	19 days/3 years	>100	0	5.8	4.2	109	Enlarged in ^{99}Tc scan	Normal	38	c.6725G>A	p.R2223H
									40	c.7006C>T	p.R2317X
Reference values		0.5–5	<10	<1 year 11–98 >1 year 2–30	6–14	80–200					

The values reflect the hormonal levels before levothyroxine substitution or after 1 month of LT4 withdrawal. ND, not determined; NI, not identified. NR not reevaluated.[a, b, and c] brothers. Patients 10, 12, 13 named as ME, RS and GD, respectively, in [37]. Patients 18 and 19 named as LD and LE, respectively, in [38]. Patients 11, 14, 15, 16, 17, and 20 named FM, PL, PC, BA, BM, and PA, respectively, in [42]. Conversion to SI units: TT_4: μg/dl × 12.87 = nmol/l, TT_3: ng/dl × 0.01536 = nmol/l, TG: ng/ml × 1.515 = pmol/l

Goiter in patients of this group was noticed as an enlarged gland in the ^{99}Tc scan in their neonatal period, and presented as a diffuse-enlarged gland without nodules latter in life. Only patient 12 presented at 4 years of age with an irregular thyroid, heterogeneous in the ultrasound study.

In this group, TT_4 serum levels were low but significantly higher from those in patients of group 1 and TT_3 levels remained in the lower normal range, also significantly different related to those of patients of group 1 that harbor TPO/DUOX2 mutations. (TT_4 μg/dl (mean ± SD) G1: 0.96 ± 0.8 vs. G2: 3.2 ± 1 ($P < 0.01$). TT_3 (ng/dl) levels (mean ± SD) (G1: 51 ± 43 vs. G2: 144 ± 40) ($P < 0.01$).

Nine out of eleven patients of this group have a normal development even when three of them (patients 16, 17, and 19) were detected late (after 2 years of age). The two handicapped children are only mildly retarded in spite of having been diagnosed at 1.3 and 4 years of age (patients 11 and 12).

The study of this group of patients found molecular disorders of the TG gene in the 11 studied patients. Patient 10 was homozygous for the p.R277X mutation, while two siblings from unrelated parents (patients 14 and 15) were homozygous for p.A2215D [37, 42].

Five patients (patients 12, 13, the brothers 18 and 19 and 20) were compound heterozygous: p.R277X/p.A2215D, p.C164Y/p.L234fsX237, p.R277X/p.R1511X, and p.R2223H/p.R2317X, respectively. The p.L234fsX237, p.R277X, p.R2317X, and p.R1511X mutations result in grossly truncated proteins with limited but not absent ability to generate thyroid hormone, the p.C164Y mutation interferes with the formation of disulfide bridges disrupting the tertiary structure of TG and the p.A2215D and p.2223H mutations may cause structural instability leading to deficient TG export [37, 38, 42].

Finally, the three remaining patients (11, 16, and 17) were found heterozygous for p.R277X with only one mutated allele detected [42].

A detailed description of the molecular aspects of each patient from both groups is available in our previous published studies [13, 17, 21, 37, 38, 42].

Discussion

Here, we describe the characteristics of a large group of CH patients with goiter and suspected dyshormonogenesis in whom the molecular approach helped to establish the etiology of the thyroid disorder. All subjects were found to carry mutations of different genes involved in iodination or synthesis of TG, that are the main currently known causes of hereditary inborn errors in thyroid hormone synthesis.

The organification process occurs in the thyroid, where iodide is rapidly oxidized and bonded to tyrosyl residues in TG [26]. The process requires the presence of iodide, TPO, a supply of H_20_2 (DUOX system), and an iodine acceptor protein (TG). Disorders of this complex mechanism include the absence or reduction of TPO activity as has been described in cases of total or partial iodide organification defects (TIOD or PIOD). The increased discharge of iodide after the administration of potassium perchlorate in the absence of thyroid antibodies has been the gold standard method to assess defects in thyroid hormone organification. The inability to organify iodide leads to its release and alters its availability to be used as substrate for thyroid hormone synthesis [5].

Consequently, our patients in group 1 suspected for organification defects were unable to produce thyroid hormone. High TSH with very low TT_4 and low or normal low TT_3 were consistent findings in this group. Goiter was present since birth as evidenced by the thyroid ^{99}Tc scan and in older patients appeared as a big thyroid gland prone to develop nodules. All patients in this group had a very high PDT.

Mental retardation was important in those patients with delayed diagnosis and the impact of thyroid deficient supply seemed to impact since birth, as children detected in early infancy also showed retardation.

The measurement of TG serum concentration, which was consistently very high in this group, also represented an important diagnostic tool. Although not fully explained, the enormous increment of its serum levels in the organification defects could be related to the availability of poorly iodinated TG in the apical follicular membrane to be capture by the megaline receptor protein and transported to the basal membrane by transcytosis [26].

Iodide organification defects are associated with inactivating mutations in TPO gene and in the past 2 decades at least 50 inactivating mutations in this gene have been reported [6–18]. In our population, out of the nine patients that underwent TPO gene studies, 6 were fully or partially characterized. Two were compound heterozygous and four had only one mutated allele of TPO gene [13, 17]. Overall, exon 8 was the most affected region of the TPO gene in our studied population.

In those patients with only one mutation found the sequenciation of all exons, exon/intron boundaries and regulatory regions of TPO and DUOXA2 genes, and SSCP analysis of the 33 exons of DUOX2 gene was unfruitful. Moreover, the haplotyping of the TPO alleles performed using informative polymorphisms in the coding regions excluded the possibility of major deletions in one allele. Thus, the apparent absence of the second mutation could probably be explained by technical limitations of the direct sequencing and haplotype analysis (e.g. deletion of a single

exon that does not contain an informative polymorphism, small deletions between the polymorphic sites, or a mutation in distant regulatory regions of the TPO gene or in remote intronic regions).

TPO has an absolute requirement of H_2O_2 which acts as an electron acceptor. H_2O_2 is generated on the apical plasma membrane of the thyroid follicular cell by a metabolic pathway involving members of the DUOX system: DUOX1, DUOX2, DUOXA1, and DUOXA2.

SSCP analysis of the DUOX2 gene and sequencing of the fragments presenting aberrant migration patterns revealed DUOX2 defects in three patients from two unrelated families [21]. In patient 7, that had two different mutations in exons 2 and 21 the PDT was 46% (the lower of this group) pointing out some residual activity of DUOX2. Organification disorders in the brothers 8 and 9 produced a 60 and 68% of perchlorate discharge. In spite of the presumed severe defect, the older sibling already as an adult presents only mild mental retardation. As she has been diagnosed and treated at 8 months of life, probably a moderate defect in thyroid hormone availability can be presumed [21].

A recent French communication on CH patients detected between 2003 and 2006, found that 38.8% (71/183) had a normally located thyroid gland, and 25 out of the 71 (35%) showed an abnormal PDT. Hypothyroidism was thought to be more severe in patients with higher discharge who might have been affected in uterus and probably harbor mutations for the organification machinery [48]. No molecular studies, however, were performed to substantiate the accuracy of this diagnostic approach.

Based on the abnormality of PDT, the authors define TIOD as PDT $>$ 90% and PIOD (PDT $<$ 90 $>$10%). The authors speculate that the former may suffer from disorders in the key enzymes of organification, the later may be affected as well by other underlying mechanisms as delayed or diminished activity of enzymes involved in thyroid hormone production, or defects in iodine storage and release.

In our nine patients of G1, only one subject would correspond to TIOD and in this patient we were able to identify only one mutation for TPO. The remaining eight patients had PTD ranging from 46 to 90% also with consistent biochemical findings of severe permanent hypothyroidism. As suggested, these disorders may be considered in a wide spectrum of defects that reflects the molecular impact at protein level of different mutations even in the heterozygous state.

The patients of group 2 were studied for defects in TG synthesis. Up to now, 51 different inactivating mutations have been discovered in individuals with congenital goiter. Mutations described have been classified as missense and nonsense mutations, frameshift mutations by single nucleotide deletion or insertion and splicing mutations in the exonic or intronic consensus sites [26–44]. The reported clinical spectrum of phenotypes ranges from mild to severe goitrous hypothyroidism. Our patients were mainly detected by neonatal screening and 6 were treated in the first days of life. The remaining 4 were detected between 1.3 and 4 years of age and, in spite of late treatment have normal development or mild retardation. In this group, serum TT_4 1evels at diagnosis were low but significantly higher than those of group 1 and the same happened with TT_3 levels. This finding was explained by Kanou et al. as the possible expression of an increased thyroidal type 2 iodothyronine deiodinase in patients with defective TG expression [39]. Serum TG concentration was very useful in the characterization of this group as serum TG was low in relation to the degree of TSH stimulation suggesting the presence of TG synthesis defects. The absence of iodide discharge in the perchlorate test in these patients completed the figure for the suspected defect.

In our analysis, eight patients were fully characterized and three carried only one mutation heterozygous [37, 38, 42]. The same considerations as we speculated for the TPO gene mutations could take account in this case. Exons 7 and 38 might be considered as hotspots for loss of function in the TG gene in our population and p.R277X the most prevalent finding.

In our cohort of patients, familiar occurrence was present in four families. In most of them, the finding of a new baby affected and characterized prompted the localization and diagnosis of the older sibling. In others, the full characterization of the first affected child allowed the early identification of the new affected baby and for an accurate molecular study and early treatment.

In countries where resources for molecular technology are not fully available, the identification of patients and their characterization is the first step for the assessment of familiar thyroid disorders.

When a newborn or a child is diagnosed with hypothyroidism, the treatment is prompted without delay, and when permanent disease is confirmed, permanently maintained. At the confirmation step as a newborn or later in childhood, the information given by biochemical and imaging assessment followed by molecular findings alerts the family on familiar occurrence. Moreover, the communication on transient conditions associated with some molecular defects in DUOX2 mutations occurring with bi or monoallelic findings points out the need of identification of the families harboring these mutations because they may be faced with different scenarios when their babies are screened for CH [19]. On the other hand, patients that prevented mental retardation may transmit in the future the disease to their own children.

In summary, the clinical and biochemical characterization of patients with CH and goiter and the strategy used to establish the etiology helped to better define our patient

population and to optimize molecular analysis that are limited in our environment. Nevertheless, our findings and further investigations on genotype–phenotype relationships will probably help to explain differences in these patients outcome and allowed us to develop new diagnostic and therapeutic strategies for the adequate counseling and care of these patients.

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