Title

Congenital Hypothyroidism in Two Sudanese Families harboring a novel Iodotyrosine deiodinase mutation (IYD R279C)

Running Head

Novel IYD Defect in 2 Sudanese Families

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Key Words

Dehalogenase, iodine deficiency, monoiodotyrosine, congenital hypothyroidism, iodotyrosine deiodinase, goiter

Abstract: *(129 words)*

Background: Congenital hypothyroidism due to defects in iodotyrosine deiodinase has variable phenotypes and can present as hypothyroid or with normal thyroid testing.

Methods: Whole exome sequencing was performed in individuals from 2 families originating from different regions of Sudan. Mass spectrometry of urine and serum iodotyrosines was performed on subjects from both families.
Results: A novel iodotyrosine deiodinase (IYD) mutation (c.835C>T; R279C) was identified in individuals from 2 Sudanese families inherited as autosomal recessive. The mutation was identified by multiple in silica analyses to likely be detrimental.
Serum and urine monoiodotyrosine and diiodotyrosine were markedly elevated in the homozygous subjects.
Conclusion: Measurement of serum and urine diiodotyrosine and monoiodotyrosine was more sensitive than urine iodine or serum thyroid function tests to determine the effect of the IYD mutation.

Introduction

Congenital hypothyroidism (CH) affects 1:3000 newborns. A delay in diagnosis and treatment may result in significant growth and developmental retardation as well as severe intellectual impairment. Thyroid dysgenesis is the cause of 65% of CH, while dyshormonogenesis and gland-in-situ accounts for 35% of CH [1]. Iodine is an essential component of thyroid hormone (TH) synthesis and when iodine is limited in supply TH synthesis is impaired. Assurance of sufficient supply of iodine for TH synthesis depends on: (1) the environmental supply of iodine; (2) the ability of the thyroid to transport iodine into the thyroid cells; (3) sufficient recycling of iodotyrosines so that maximum efficiency of iodine utilization occurs. Multiple genes are involved in thyroid hormone synthesis and specifically iodine metabolism [2]. Release of T_4 and T_3 from the thyroid gland requires proteolysis of thyroglobulin (TG), during which monoiodotyrosine (MIT) and diiodotyrosine (DIT) are also released. The iodine of MIT and DIT cannot be used directly and require deiodination by the enzyme iodotyrosine dehalogenase 1 (IYD). IYD allows iodine to be recycled to synthesize more T_3 and T_4 [3]. When environmental iodine is not in abundance, the presence

of functional IYD becomes more important in conservation and utilization of iodine in the synthesis of TH.

We report 2 Sudanese families from different parts of the country with CH and goiter due to a novel mutation in the *IYD* gene. The phenotype as determined by thyroid function tests and goiter were variable in the 2 families ranging from hypothyroid to euthyroid despite the same genetic defect. Whereas urine iodine concentration was unable to predict the degree of iodine deficiency in these patients, LC-MS-MS analysis of serum and urine confirmed excessive accumulation of MIT and DIT in the affected patients. These families illustrate the utility of measurement of MIT and DIT in diagnosing CH in iodine sufficient and iodine borderline areas.

Methods

All patients were referred to a pediatric endocrinologist at the University of Khartoum, Sudan presenting with stigmata of hypothyroidism or due to a sibling having hypothyroidism. Oral consent from patients or their guardians and their family members was obtained prior to blood sampling due to literacy. Studies were approved by the University of Miami Institutional Review Board. The initial thyroid testing (TSH and FT_4) was done in the Sudan at the time of diagnosis. When the patients came for follow up at their local clinic in the Sudan, blood was then sent to Miami, Florida. Testing was performed using the Immulite® 1000 (Siemens, Munich, Germany) platform as previously described [4]. Isolation of genomic DNA from whole blood using the Qiagen QIAamp® DNA Blood Mini Kit (Hilden, Germany) were carried out at the University of Miami. Blood samples were obtained from propositus, siblings and parents when possible. For each of the 2 families, gDNA from the proband along with one parent was submitted to whole exome sequencing (WES) (Novogene, Agilent SureSelect Human All Exon V6 Kit). A compilation of thyroid genes linked to thyroid disorders was evaluated and possible mutations linked to the phenotype were identified based on predicted functional scores, allele frequency, and zygosity [4]. Then gDNA from all family members available were analyzed and confirmed by Sanger sequencing (Genewiz, Abi 3730xl DNA Analyzer) to

verify the WES results and establish the genotype of all sampled family members . All identified variants were further evaluated by in-silico prediction scores [5-9]

Measurement of MIT and DIT in serum and urine was performed by liquid chromatography coupled to tandem mass spectrometry [10]. Iodine was quantified by the Sandell-Kolthoff method [11].

Case Presentations and Results

Family 1.

Family 1 (Fig. 1A) from the Western Sudan was identified in 2017 when the propositus, from consanguineous parents (first cousins), presented at age 7 with impaired cognitive function, goiter and found to have elevated TSH and low serum T_4. He was started on levothyroxine (L-T_4) and prompted evaluation of a younger brother aged 8 years and a younger sister aged 3 years. Both siblings had what was considered to be “low IQ” by the clinician with goiter on exam and thyroid function tests confirmed tests similar to the propositus with high serum TSH and low serum T4 with classic hypothyroid facies of periorbital edema and swollen lips (Fig 1B). Serum was obtained 7 days after starting L-T_4 therapy and again after 6 months and sent to Miami for further analysis (Fig 1C). By 7 days of treatment thyroid tests of the propositus (II-1) and his brother (II-2) normalized, although those of the younger sister (II3) did not normalize until 6 months (Fig 1A). WES carried out on the propositus and his mother demonstrated a mutation in exon 5 (c.835C>T; R279C) of the *IYD* gene. Sanger sequencing confirmed that all 3 siblings (II-1, II-2, II-3) where homozygous for the mutation while the parents were heterozygous (Fig 1D).

Due to the discovery of the *IYD* gene mutation, L-T_4 supplementation was discontinued and the 3 siblings were treated with Lugol’s iodine 2 drops twice daily for 4 weeks and repeated thyroid tests confirmed maintenance of euthyroidism (SupplementalTable). After 4 weeks, due to difficulty in obtaining the Lugol’s iodine, the patients were switched back

to L-T_4 with continued maintenance of normal thyroid tests. Spot urine iodine was obtained after restarting L-T_4 (Fig. 1A).

Family 2.

Family 2 from the Northern Sudan was identified in 2018 when the propositus (II-1, Fig 2), born to consanguineous parents (first cousins) presented at age 7 and was placed on L-T_4. Available medical notes were incomplete. At age 13 years he self-discontinued L-T_4, felt well, his thyroid function tests were normal and he did not resume L-T_4 treatment. Siblings II-2 and II-3 had goiter and were not treated with L-T_4 but II-4 who did not have a goiter had some lethargy and was briefly treated with L-T_4. WES carried out on DNA from the propositus and mother demonstrated the same IYD mutation as that found in Family 1, (exon 5; c.835C>T; R279C) with subjects II-1, II-3, II-4 homozygous and the mother (I-2) and a sibling (II-2) heterozygous. The father was deceased. Spot urine iodine was obtained (Fig. 2). Blood and urine was obtained on two different occasions and sent to Miami for analysis with similar normal values for all thyroid function tests and urine iodine. Due to the identical mutation identified in these 2 families from different parts of the Sudan the gene locus was haplotyped demonstrating that the minimally shared genetic gene interval surrounding the mutation was 2.7 megabases (Supplemental Fig 1).

Iodothyronine Concentrations in Serum and Urine

Iodothyronine measurements were performed in the serum from individuals of Family 1 (Fig. 1A) and urine and serum of individuals from Family 2 (Fig 2). Serum MIT and DIT were markedly elevated in the serum of all homozygous individuals compared to the heterozygous father (I-1) and T_3 and T_4 concentrations were slightly higher in the affected subjects. In family 2, serum MIT and DIT were markedly elevated in the affected individuals, where as T_3 and T_4 were not. However, urine MIT and DIT were markedly elevated in the affected individuals.

Discussion

A novel mutation (c.835C>T; R279C) in the IYD gene was identified in 2 Sudanese families from different regions of the Sudan manifesting autosomal recessive inheritance. Multiple *in silico* analyses (SIFT, Polyphen2 HVAR, Polyphen 2 HDIV, Mutation Tester, Mutation Assessor, FATHMM and CADD) indicated that the mutation likely had a detrimental consequence.

The first reported patient with a dehalogenase defect, confirmed by measurement of iodotyrosines, was described in 1955 [12]. The patient was born with a goiter in an area of Holland where goiter was endemic. He was treated with "thyroid powder" at 9 months of age with shrinkage of the goiter. A younger brother who was not treated with "thyroid powder" remained goitrous and mentally delayed. The parents were unrelated and had no goiter. A series of elegant experiments demonstrated that intravenously administered I^{-131} labeled *dl*-DIT and *dl*-MIT were excreted in the urine almost entirely unchanged. The authors concluded that there was a defect in *dehalogenase* [13]. While other cases were reported earlier this was the first to directly demonstrate altered metabolism of iodotyrosines. Subsequently additional cases of presumptive *dehalogenase* defects were identified [14].

However, it wasn't until 50 years later when *dehalogenase 1*, or iodotyrosine dehydrogenase (IYD) was sequenced and characterized [15]. Since then 4 unique IYD mutations have been reported in 9 individuals [16-18] (Fig. 3). There is significant phenotypic variability in these patients even in those with the identical mutations in different families as described in the present report with a novel 5th unique IYD mutation. TSH was variably elevated in 3 individuals prior to starting $L\text{-}T_4$ treatment (Patients 1,4,8, Fig. 2) and in 1 individual (Patient 7, Fig. 3) the TSH ultimately became elevated 5 years after initial diagnosis. Age of diagnosis and mode of inheritance was also variable (Fig. 2). MIT and DIT were consistently elevated. The loss of iodotyrosines in urine reduces the iodine available for recirculation producing iodine deficiency. When the availability of environmental iodine is limited, loss of the iodotyrosines lowers the threshold for hypothyroidism. To this point, in Family 1 reported above, replacement of iodine

with Lugol's iodine solution was able to substitute for L-T_4 and maintain normal thyroid tests. In summary, measurement of serum and urine DIT and MIT was more sensitive than urine iodine or thyroid function tests to identify a defect in iodotyrosines deiodination and the presence of a IYD mutation.

Authors' Contributions:

Reham Shareef – Identification of family in Sudan and collection of specimens
Aryel Furman – Haplotyping and review of manuscript
Ryan Bruellman – Genotyping and identification of the gene in Family 2
Yui Watanabe – Genotyping and identification of the gene in Family 1
Mohammed A Abdullah – Supervising collection of samples in Sudan
Alexandra Dumitrescu – Review of manuscript and intellectual input into the design of the study
Samuel Refetoff – Intellectual input into the design of the study and review of the manuscript.
Marco Borsò- Mass spectrometry analysis
Andrea Bertolini- Mass spectrometry analysis
Alessandro Saba- Mass spectrometry analysis
Riccardo Zucchi- Mass spectrometry analysis and intellectual input into the study and review of the manuscript.
Roy E. Weiss – Conceived the study, intellectual input into design and raised funds for the study, writing of the manuscript.

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Statement of Ethics

The authors have no ethical conflicts to disclose. The subjects agreed to participate in the study. The study was approved by the Institutional Review Board at the University of Miami Miller School of Medicine protocol number 20140632

Author Disclosure Statement

REW is on the Advisory Board and scientific cofounder for PriZm Therapeutics and on the Advisory Board of FBIIO Acquision Corp XXV. The other authors have no competing interests.

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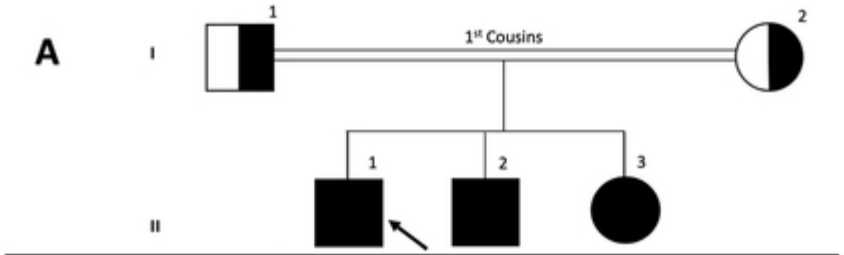
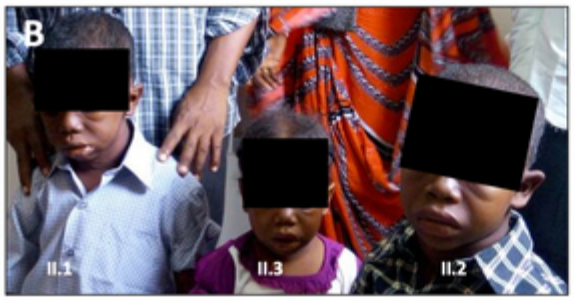
Figures:

Fig. 1. Pedigree, thyroid function tests and urine iodine values of Family 1
(A). Photograph of II-1, II-2, and II-3 from Family 1 at the time of diagnosis (B) with facial features typical of hypothyroidism with periorbital edema and swelling of the lips; and 6 months after initiation of L-T4 therapy (C). Sanger sequencing chromatograms (D) confirming the IYD mutation in Exon 5 R279C. Values in Red are above the limit of normal and values in Blue are below the limit of normal. UIC, urine iodine concentration. Treatment a, = 7 days after starting L-T_4; b, 6 months after starting L-T_4.



	I-1	II-1		II-2		II-3		I-2	
Age (at diagnosis)	30y	10y	11y	8y	9y	3y	4y	25y	26y
Treatment	-	L-T4 [a]	L-T4 [b]	L-T4 [a]	L-T4 [b]	L-T4 [a]	L-T4 [b]	-	-
Goiter	-	+	-	+	-	+	-	-	-
TT4 *µg/dL (4.5-12.5)*	9.82	14.6	9.14	17.3	7.94	12.8	10.8	9.95	
TT3 *ng/dL (81-178)*	82.5	272	123	408	105	529	125	97.8	
FT4 *ng/dL(0.89-1.76)*	1.37	2.29	1.53	2.96	1.84	2.18	1.51	1.52	
TSH *µIU/mL (0.4–4.0)*	3.66	2.07	<0.004	1.78	0.021	18.8	0.576	3.98	
TBG *µg/mL (14-31)*	24.9	41.9	25.5	21.8	15.5	35.4	29.7	22.2	
TG *ng/mL (1.7-55.6)*	10.6	849	6.70	5,880	59.9	944	18.7	9.09	
***s* MIT** *ng/ml (0.08-08)*	0.075	2.8		6		35		0.35	
***s* DIT** *ng/ml (<0.3)*	0.2	0.95		8		7.5		0.6	
UIC *µg l/L (34-523)*			319		86		65		59



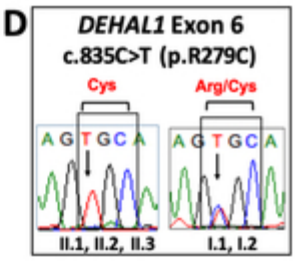
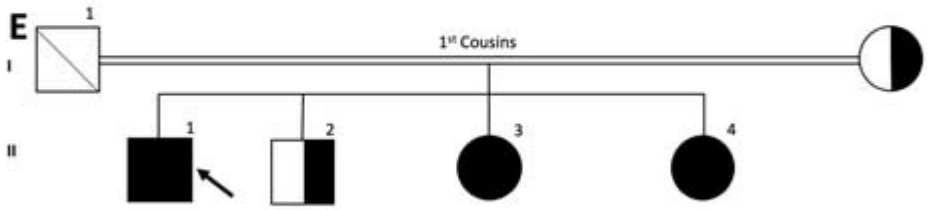


Fig 2. Pedigree, thyroid function tests and urine iodine values of Family 2. Values in Red are above the limit of normal and values in Blue are below the limit of normal. Treatment a, = 7 days after starting L-T_4; b, 6 months after starting L-T_4.

Fig. 2



	II-1	II-2			II-3			II-4			I-2		
Age (at diagnosis)	22y	18y		19y	15y		16y	13y		14y	41y		42y
Treatment	-	-	-	-	-	-	-	-	-	-	-	-	-
Goiter	-	+	+	+	+	+	+	-	-	-	-	-	-
TT4 *µg/dL*	7.69	8.31	5.73		6.55	6.41		8.19	6.57		9.03	8.01	
TT3 *ng/dL*	106	156	109		126	109		139	124		83.0	96.6	
FT4 *ng/dL*	1.93	1.02	1.09		1.88	1.39		1.2	1.17		2.5	1.23	
TSH *µIU/mL*	2.23	1.22	1.30		1.94	1.85		2.22	1.86		1.81	1.53	
TBG *µg/mL*	12.6	28.3	26.3		13.8	18.6		25.4	19.8		10.6	22.0	
TG *ng/mL*	40.8	19.0	13.6		0.477	1.21		27.6	20.2		12.4	11.7	
Tg Ab *IU/mL*	<20	<20	<20		>3,000	>3,000		<20	<20		<20	<20	
s MIT *ng/ml*	2	3			10			08			1.8		
***s* DIT** *ng/ml*	1.5	1.5			1.5			5			0.4		
***u* MIT / *u* DIT** (0.8-1.2 / 0.2-0.5)		7/5			70/110			50/55			-		
UIC *µg l/L*				143			91			125			170

Fig 3. Comparison of phenotype and genotype in other families with IYD gene mutations. Values in Red are above the limit of normal and values in Blue are below the limit of normal. +, present; -, not performed or data not available.

Patient	1	2	3	4	5	6	7		8	(9)
Mutation	p.R101W	p.F105-I106L		p.I116L	p.A220T					
	Homo	Homo	Homo	Homo	Homo	Homo	Hetero		Homo	Homo
Diagnosis	1.5y	infancy	infancy	8y	5y	infancy	(9y)	14y	15.9y	(6y)
L-T4 at TFTs	-	-	-	-	+	+	-	-	-	-
TSH	390	-	-	139	1.2	0.6	0.5	>100	1400	2.2
TT3 ug/dL	70	-	-	-	192	124	146	-	-	-
FT3 pg/mL	-	-	-	1.4	-	-	2.25	2.6	2.9	4.2
TT4 ug/dL	1.4	-	-	-	7.4	10.1	9.7	-	-	-
FT4 ng/dL	-	-	-	<0.2	0.5	1.2	1.1	<0.3	<0.5	1.4
TG	-	-	-	-	1040	<20	110	-	6800	11.7
U-DIT, MIT	-	-	-	-	high	normal	high	-	-	high
S-DIT	high	-	-	high	-	-	-	-	-	-
Goiter	+	+	+	+	+	+	None	+	+	None
Reference	Moreno J.C et al., NEJM, 2008 [17]				Afink G. et al., JCEM, 2008 [15]				Burniat A. et al., JCEM, 2012 [16]	