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BRIEF REPORT

Congenital Hypothyroidism due to Oligogenic Mutations in Two Sudanese Families

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Dyshormonogenic congenital hypothyroidism (CH) generally results from biallelic defects in thyroid hormone synthesis genes. Whole exome sequencing allows easier identification of multiple gene defects. Two Sudanese families with CH resulting from oligogenic defects identified by whole exome sequencing are presented. In family 1, the proposita with CH and goiter was heterozygous for three *TPO*, one *TG*, and one *DUOX2* mutations, including three novel variants inherited from both parents. In family 2, two brothers with psychomotor delay and goiter were homozygous for digenic mutations in the *DUOX2* and *DUOX1* genes, while their asymptomatic parents were heterozygous. Accumulation of pathogenic mutations may contribute to CH.

Keywords: *TPO* gene, *TG* gene, *DUOX2* gene, *DUOX1* gene, oligogenic mutations, congenital hypothyroidism

Introduction

Until recently, the cause of dyshormonogenic congenital hypothyroidism (CH) was identified in individuals with biallelic mutations in single genes (1). Rarely, CH has been explained by digenic or oligogenic mutations independent of whole exome sequencing (WES) (2–4). Two Sudanese families with oligogenic variants resulting in CH are reported.

Patients

Family 1

The proposita was a west Sudanese three-year-old female who presented with goiter and hypothyroidism (thyrotropin [TSH] >100 μIU/mL) at the age of 10 months. There is no neonatal screening for CH in the Sudan. She was started on levothyroxine (LT4), producing normal growth and development. There was no family history of thyroid disease, but her parents were second cousins. Thyroid function tests (TFTs) were performed using Immulite 1000® (Siemens, Munich, Germany). LT4 treatment normalized the TSH, but thyroglobulin (Tg) was high. Other family members had normal TFTs, except the father who had slightly low total triiodothyronine (T3; Fig. 1A).

Family 2

A north Sudanese three-year-old male was diagnosed with CH at one year of age when presenting with goiter and mild psychomotor delay. He had a serum TSH of >100 μIU/mL and a total thyroxine (TT4) of 7.3 nmol/L (reference range 66–181 nmol/L). Due to this history, a younger brother had TFTs evaluated at three weeks of age, which showed a serum TSH >100 μIU/mL and a free T4 of 2.46 pmol/L (reference range 12–22 pmol/L). Both brothers were placed on LT4. Their reportedly non-consanguineous parents had no history of thyroid disease. The affected siblings had high TSH and Tg on an inadequate dose of LT4. The father had low total T3 levels (Fig. 1B).

Molecular genetics

Studies were approved by The Human Subject Research Office of The University of Miami. Written informed consent was obtained from all adult subjects and parents of the children. WES was performed on each proband and mother. The data were analyzed for autosomal recessive inheritance pattern. Fifty-three genes related to thyroid disorders were evaluated (Supplementary Table S1). The proposita of family 1 had the following heterozygous abnormalities: (i) three mutations in the *TPO* gene, one splicing variant c.483-2A>G, and two missense mutations c.1535C>A, p.P512H and c.1759G>A, p.G587R; (ii) a splicing mutation, c.5042-2A>G, in the *TG* gene; and (iii) a frameshift deletion, c.1395_1396delCC, in the *DUOX2* gene. Sanger sequencing confirmed inheritance of *TPO* G587R, *TG*, and *DUOX2* gene mutations from her father, and *TPO* P512H and c.483-2A>G

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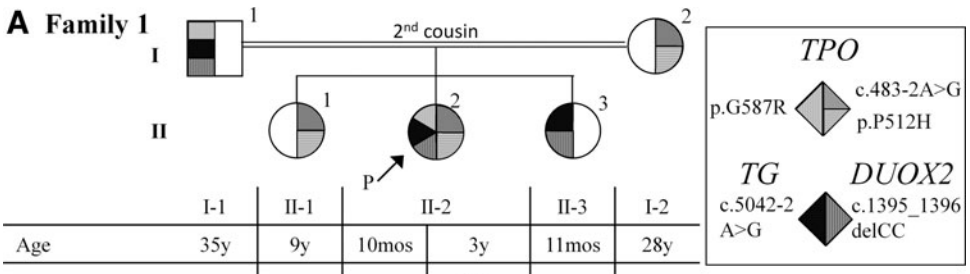
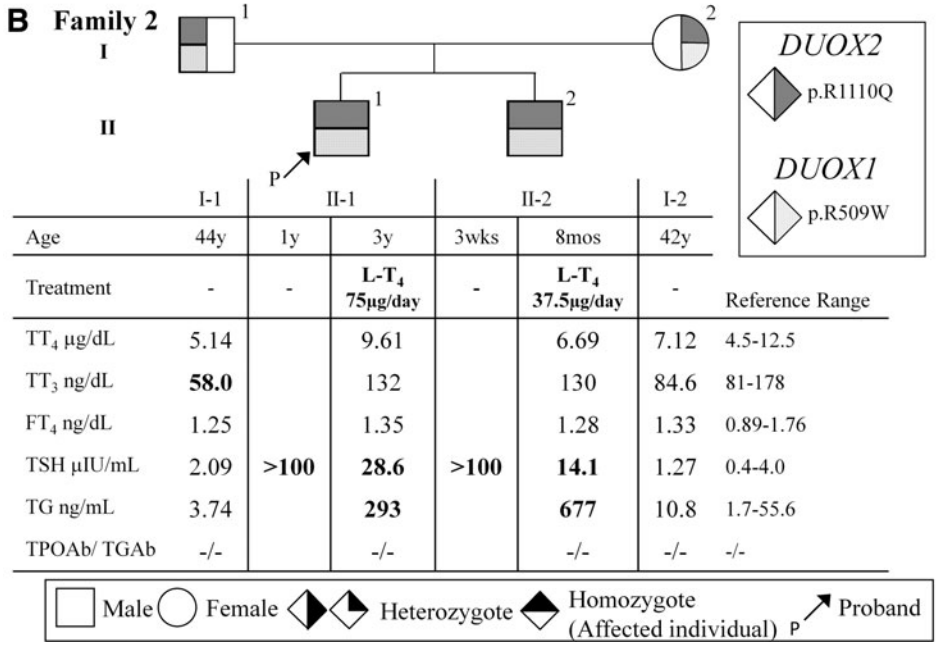


	I-1	II-1	II-2		II-3	I-2	
Age	35y	9y	10mos	3y	11mos	28y	
Treatment	-	-	-	**L-T_4 50µg/day**	-	-	Reference Range
TT_4 µg/dL	7.02	7.95		11.2	9.16	9.17	4.5-12.5
TT_3 ng/dL	**71.2**	99.6		122	148	109	81-178
FT_4 ng/dL	1.24	1.33		1.55	1.24	1.17	0.89-1.76
TSH µIU/mL	0.44	1.13	**>100**	0.47	2.21	0.84	0.4-4.0
TG ng/mL	2.70	5.02		**242**	21.1	5.57	1.7-55.6
TPOAb/ TGAb	-/-	-/-		-/-	-/-	-/-	-/-



	I-1	II-1		II-2		I-2	
Age	44y	1y	3y	3wks	8mos	42y	
Treatment	-	-	**L-T_4 75µg/day**	-	**L-T_4 37.5µg/day**	-	Reference Range
TT_4 µg/dL	5.14		9.61		6.69	7.12	4.5-12.5
TT_3 ng/dL	**58.0**		132		130	84.6	81-178
FT_4 ng/dL	1.25		1.35		1.28	1.33	0.89-1.76
TSH µIU/mL	2.09	**>100**	**28.6**	**>100**	**14.1**	1.27	0.4-4.0
TG ng/mL	3.74		**293**		**677**	10.8	1.7-55.6
TPOAb/ TGAb	-/-		-/-		-/-	-/-	-/-

FIG. 1. Pedigree, thyroid function tests results, and mutations identified in family 1 (**A**) and family 2 (**B**). Generations are indicated with Roman numerals, and individuals with Arabic numbers above each symbol. Laboratory data are aligned below each symbol. Vertical shading of symbols indicates heterozygous for the mutation and horizontal shading of the symbols indicates homozygosity for the mutation. Abnormal values are in bold type. LT4, levothyroxine; TT4, total thyroxine; TT3, total triiodothyronine; fT4, free thyroxine; TSH, thyrotropin; Tg, thyroglobulin; TPOAb, thyroid peroxidase antibody; TgAb; thyroglobulin antibody. International System of Units: TT4, μg/dL = 12.87 nmol/L; TT3, ng/dL = 0.0154 nmol/L; fT4, ng/dL = 12.87 pmol/L; TBG, μg/mL = 0.0185 μmol/L.

from the mother (Fig. 1A). The missense variants are predicted by an *in silico* algorithm to be deleterious (Supplementary Table S2). Protein modeling *in silico* was performed and all reported mutations result in significant protein conformational changes (Supplementary Fig. S1). They were previously reported in The Genome Aggregation Database (gnomAD). However, the clinical significance was unknown. The splicing and frameshift variants were not present in gnomAD, dbSNP, and the Exome Variant Server. Both splicing variants located in the acceptor splice site are predicted to affect splicing (Supplementary Tables S2–S4). The *DUOX2* frameshift variant results in a premature stop at amino acid 514 (p.Q466fsX48).

WES of the propositus of family 2 identified a biallelic missense mutation resulting in *DUOX2* c.3329G>A, p.R1110Q, inherited digenically with a homozygous missense mutation resulting in *DUOX1* c.1525C>T, p.R509W. Sanger sequencing confirmed that affected siblings were homozygous, while their parents were heterozygous (Fig. 1B). The *DUOX2* variant is a known pathogenic mutation producing CH (5). The *DUOX1* mutation was reported in the gnomAD and predicted to be deleterious (Supplementary Table S2), but its clinical significance was unreported.

Discussion

The proposita of family 1 harbors three *TPO* and one mutation in each *TG* and *DUOX2* genes. Interestingly, no hypothyroidism was found in other family members with two or three variants. This case supports that sporadic CH may result from combination of multiple gene mutations (2–4). Dissecting the contribution of each mutation to the phenotype would require complex functional studies. Regarding findings in family 2 with severe CH, digenic *DUOX2* and *DUOX1* mutations have been reported recently (6). A case from Japan with *DUOX2* R1110Q had mild hypothyroidism

(5) compared to the CH of the present case. However, *DUOX1* mutations and environmental factors such as iodine intake that might contribute to clinical differences were not assessed. Even though expressed at a lower level (1), DUOX1 likely compensates for DUOX2 deficiency affecting the disease severity (6).

In conclusion, the accumulation of pathogenic mutations in several genes may contribute to the pathophysiology of CH. WES allows the identification of defects in multiple genes that, in combination, may be responsible for congenital dyshormonogenesis previously believed to be single gene recessive defects. Thus, digenic or oligogenic mutations may result in CH, a mechanism diverging from a simple autosomal recessive model.

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Author Disclosure Statement

No competing financial interests exist.

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