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Case Report

A Novel c.554+5C>T Mutation in the DUOXA2 Gene Combined with p.R885Q Mutation in the DUOX2 Gene Causing Congenital Hypothyroidism

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ABSTRACT

The coexistence of mutations in the dual oxidase maturation factor 2 (*DUOXA2*) and dual oxidase 2 (*DUOX2*) genes is rarely identified in congenital hypothyroidism (CH). This study reports a boy with CH due to a novel splice-site mutation in the *DUOXA2* gene and a missense mutation in the *DUOX2* gene. A four-year-old boy was diagnosed with CH at neonatal screening and was enrolled in this study. The *DUOXA2*, *DUOX2*, thyroid peroxidase (*TPO*), and thyrotropin receptor (*TSHR*) genes were considered for genetic defects screening. Genomic DNA was extracted from peripheral blood leukocytes, and Sanger sequencing was used to screen the mutations in the exon fragments. Family members of the patient and the controls were also enrolled and evaluated. The boy harbored compound heterozygous mutations including a novel splice-site mutation c.554+5C>T in the maternal *DUOXA2* allele and c.2654G>A (p.R885Q) in the paternal *DUOX2* allele. The germline mutations from his parents were consistent with an autosomal recessive inheritance pattern. No mutations in the *TPO* and *TSHR* genes were detected. A novel splice-site mutation c.554+5C>T in the *DUOXA2* gene and a mutation p.R885Q in the *DUOX2* gene were identified in a 4-year-old patient with goitrous CH.

Keywords: Congenital hypothyroidism, dual oxidase maturation factor 2, dual oxidase 2, mutation

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WHAT IS ALREADY KNOWN ON THIS TOPIC?

The dual oxidase maturation factor 2 (*DUOXA2*) and dual oxidase 2 (*DUOX2*) genes are rarely identified in congenital hypothyroidism (CH).

WHAT THIS STUDY ADDS?

We detected a novel splice-site mutation in the *DUOXA2* gene and a missense mutation in the *DUOX2* gene in a boy with CH.

Introduction

It is generally known that congenital hypothyroidism (CH) is the most common neonatal endocrine disorder and occurs in approximately 1:2000-1:4000 of newborns. CH cases are caused by various defects including thyroid dysgenesis and thyroid hormone synthesis defects (1,2). Previous studies revealed

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inactivating mutations in a specific subtype of CH. Of the known genes, mutations in dual oxidase maturation factor 2 (*DUOXA2*), dual oxidase 2 (*DUOX2*), thyroid peroxidase (*TPO*), and thyrotropin receptor (*TSHR*), that are all known to be related to thyroid dysgenesis or dyshormonogenesis and that are all inherited in an autosomal recessive pattern, have been reported (3,4,5,6).

The existing data suggest that inactivating mutations in the TSHR gene are responsible for thyrotropin (TSH) resistance and thyroid dysgenesis (1,2,6,7). Mutations in the *DUOXA2*, *DUOX2*, and *TPO* genes are responsible for thyroid dyshormonogenesis and goitrous congenital hypothyroidism (GCH) (3,4,5). Defective thyroid hormone synthesis represents most cases of GCH. Mutations in the *DUOXA2, DUOX2, TPO*, and *TSHR* genes are more common than those in thyroglobulin (TG) and paired box 8 (PAX8) genes in CH (1,2).

Currently, it is believed that H_2O_2 generation needs the catalytic core of *DUOX2*. Oxidation reaction is crucial for the iodination of TG during thyroid hormone synthesis. *DUOXA2* is required for normal *DUOX2* enzymatic activity. It has been identified that *DUOXA2* is crucial for *DUOX2* maturation, and genetic defects in *DUOX2* cause CH and subclinical another important candidate gene for CH and SCH (3,4,8,9,10).

To date, the genetic defects in CH have not been fully understood. In this study, the *DUOXA2*, *DUOX2*, *TPO*, and *TSHR* genes were considered for screening genetic defects in a male patient with GCH reported below.

Case Report

A four-year-old boy came from the city of Suqian in Jiangsu Province, China. He had been diagnosed as CH at the neonatal screening and treatment was initiated. He was recruited by our team for investigation of a possible mutation. The patient was born to non-consanguineous parents without thyroid disease. CH was diagnosed on the basis of serum TSH, free thyroxine (fT_4), and free triiodothyronine (fT_3) levels. Daily L-thyroxine was administered to the patient at diagnosis. Thyroid gland examinations were performed with ^{99m}Tc thyroid scan and ultrasound at age four years. A total of 105 unrelated healthy controls were enrolled in this study. This study was approved by the ethics committee of the hospital. Written informed consent was obtained. Blood samples were collected from the participants.

At the beginning of the study, venous blood samples were obtained from the boy. DNA was extracted from peripheral blood leukocytes. Primers were designed to target the flanking intron regions of the exons. All exons of the *DUOXA2* (MIM# 612772, GenBank NM_207581.3), *DUOX2* (MIM# 606759, GenBank NM_014080.4), *TPO* (MIM# 606765, GenBank NM_000547.5), and *TSHR* (MIM# 603372, GenBank NM_000369.2) genes were amplified by polymerase chain reaction (PCR). The amplified PCR products were Sanger sequenced directly for variance analysis. All exons of the above genes were first amplified in the patient. If a mutation was identified, the target fragment was also amplified in the patient's parents and in 105 control individuals. Novel mutations were analyzed by bioinformatic tools.

The clinical summary and thyroid function of the boy and his parents are shown in Table 1. The proband had overt CH at neonatal screening. L-thyroxine was the treatment of choice at diagnosis, with a starting dose of 10 μ was the treatment of choice at diagnosis, with a starting dose of 10s are shown in Table 1. The proband had. Thyroid function tests showed that the parents had normal thyroid function. Thyroid ultrasound examination demonstrated enlarged thyroid lobes in our patient. Thyroid ^{99m}Tc scan revealed that the boy's thyroid appeared normally located but enlarged (Figure 1, Panel A).

As shown in Figure 2, the genetic analysis demonstrated two heterozygous mutations, a novel maternal allele splicing site variant (c.554+5C>T) (C to T substitution at position +5 of the donor site of intron 4) in the *DUOXA2* gene and another paternal allele missense mutation c.2654G>A (p.R885Q) in the exon 20 of the *DUOX2* gene, which has been reported previously (3). No mutations in the *TPO* and *TSHR* genes were detected in this study. None of the controls showed the same pathogenic variants.

The splicing site variant c.554+5C>T at the exon 4/intron 4 junction of the *DUOXA2* was not present in the Human Gene Mutation Database, nor in the dbSNP database, 1000 Genomes Project database, or PubMed. The splicing variant prediction was carried out using Human Splicing Finder, Alternative Splice Site Predictor, and SplicePort. The prediction results showed that the variant might alter gene splicing by removing the normal splice donor at the abnormal site (potential splice site: ATGgtaagc, consensus value: 92.12) or (constitutive donor: TAAAGTTCCTgtaagtatta, score: 13.255; constitutive acceptor: tgtctcccagGAATCTCCCT, score: 9.038) or (donor short sequence: ttcctgtaagta, score: 1.59992; donor short sequence: ttcctgtattaa, score: -0.995081), respectively. We concluded that the c.554+5C>T might lead to intron 4 splicing loss and altered *DUOXA2* messenger ribonucleic acid (RNA) sequence and the protein primary structure.

Table 1. Clinical and biochemical data of the family in May 2015

Variables	Normal range	Patient$^{\phi}$	Mother	Father
Age (years)	/	4	28	30
Height (cm)	/	108	160	169
Weight (kg)	/	12	55	65
Vision	/	Normal	Normal	Normal
Thyrotropin (mIU/mL)	0.34-5.44	>100.00	1.61	2.80
Free triiodothyronine (pmol/L)	2.92-5.93	2.84	3.77	4.00
Free thyroxine (pmol/L)	7.91-20.59	4.47	15.20	17.52
Thyroid size	/	Goiter	Normal	Normal

$^{\phi}$: thyroid function tests were performed at screening in the neonatal period

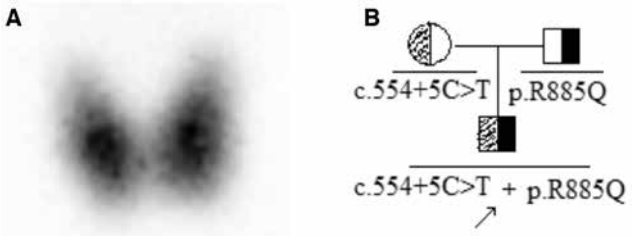

Figure 1. Thyroid ^{99m}Tc scan revealed the enlarged thyroid lobes of the boy (Panel A, anterior view). The arrow indicating the proband in the pedigree with the compound heterozygous mutations (Panel B)

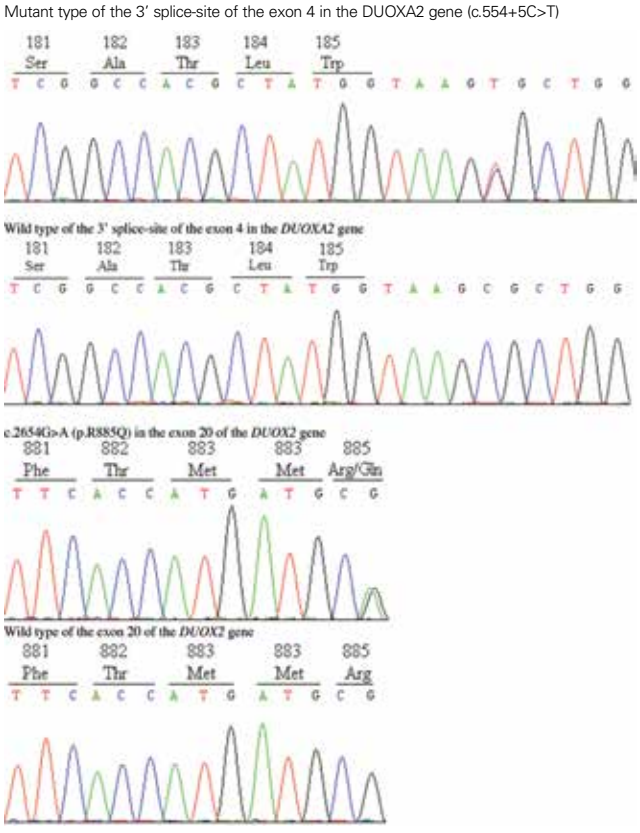

Figure 2. The genotypes revealing the heterozygous mutations in the *DUOXA2* gene (c.554+5C>T) and in the *DUOX2* gene (c.2654G>A, p.R885Q)

Discussion

The present study demonstrated compound heterozygous mutations, c.554+5C>T in the *DUOXA2* gene and c.2654G>A (p.R885Q) in the *DUOX2* gene in a pedigree with one four-year-old boy with GCH. H_2O_2 is a key element in iodine organification. *DUOXA2/DUOX2* is the main enzyme for the H_2O_2-generating system. Defects in the *DUOX2/DUOXA2* heterodimer lead to hypothyroidism and goiter. Since the first report in 2002 of *DUOX2* mutations causing CH (10,11), over 40 mutations in the *DUOX2* gene have been described correlated with CH, while only four mutations have been identified in the *DUOXA2* gene (3,8,9,10). Thus far, our splice site mutation, as far as we know, is identified for the first time as being causative of CH.

The patients with *DUOX2* or *DUOXA2* mutation show a great genotype-phenotype variability (10,11). Maruo et al (3) firstly reported the p.R885Q mutation in the *DUOX2* gene exhibiting transient hypothyroidism, which is not similar to our patient. The patient in this study had permanent CH and needed L-thyroxine replacement therapy. Heterozygous *DUOX2* gene mutations result in different phenotypes, such as transient CH, subclinical hypothyroidism, and euthyroidism. However, the coexistence of heterozygous *TSHR* and *DUOXA2* mutations causes overt hypothyroid condition (12).

Four mutations in the *DUOXA2* (p.I26M, p.Y138X, p.C189R and p.Y246X) were found to be associated with CH (3,4,8,9,10). The patient with the p.I26M, p.C189R, and p.Y138X heterozygous missense mutation in *DUOXA2* gene presented as a mild transient CH case. A homozygous nonsense mutation (p.Y246X) in patients with mild permanent CH and goiter was also identified. These patients are all of Chinese origin, indicating that this specific variant may occur at a high frequency in Chinese cohorts with CH.

Splice-site mutations are important disease-causing defects. It is estimated that approximately 10% of human genetic diseases are caused by mutations at splice sites (13). Analysis of the c.554+5C>T variation in the *DUOXA2* gene revealed that it is capable of causing disease. Possibly this is the first report of a c.554+5C>T mutation in the *DUOXA2* gene. The proband in this study presented with a normally located but enlarged thyroid gland. His parents, each with a single heterozygous mutation, both exhibited normal thyroid positioning and normal serum thyroid hormone levels. Our patient demonstrated no physical or cognitive developmental defects, primarily due to the timely and effective treatment.

Additionally, the c.554+5C>T mutation may affect the RNA transcription process and lead to genetic instability of the *DUOXA2* gene. Further comprehensive functional assessments of the detected mutation will reveal its exact mechanism in the pathogenesis of CH. The R434X mutation in the *DUOXA2* was detected by a two-stage strategy of genetic linkage studies and targeted sequencing of the candidate genes, suggesting a new testing strategy which uses next-generation sequencing in CH cases (14).

In conclusion, the present study reports a novel splicing site variant (c.554+5C>T) in the *DUOXA2* gene and another missense mutation c.2654G>A (p.R885Q) in the *DUOX2* gene. The findings indicate the importance of molecular genetic studies for the accurate diagnosis and classification of CH.

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Ethics

Ethics Committee Approval: Huai'an Second People's Hospital Ethics Committee (Approval number: 05-23-2014), Informed Consent: It was taken.

Peer-review: External peer-reviewed.

Authorship Contributions

Concept: Shao-Gang Ma, Design: Shao-Gang Ma, Data Collection or Processing: Xiao Zheng, Ya-Li Qiu, Analysis or Interpretation: Man-Li Guo, Xiao-Juan Shao, Literature Search: Man-Li Guo, and Xiao-Juan Shao, Writing: Xiao Zheng and Shao-Gang Ma.

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