Mutation spectra and genotype-phenotype analysis of congenital hypothyroidism in a neonatal population

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Abstract. Congenital hypothyroidism (CH) is a common neonatal endocrine disorder that is characterized by irreversible neurodevelopmental and growth retardation due to insufficient biosynthesis of thyroid hormones at birth. Determining the causative genetic variants in infants is important for neonatal management. It was aimed to evaluate the variant frequencies and spectrum of CH in the neonatal population of Foshan, China. A total of 105 unrelated patients with CH and 138 controls from a neonatal screening program in Foshan, China were selected. A multiplex PCR amplification-based capture panel was performed which targeted the exon regions of 30 CH-related genes. Next-generation sequencing data were processed using an in-house bioinformatics system. A total of 91 variants distributed across 16 genes were identified in 74.29% (78/105) of the patients, of which 16 were novel variants and 75 were known variants. The most frequently mutated gene was *DOUX2*, followed by *TG*, *TSHR* and *TPO*. Specifically, *DUOX2* variants p.Lys530Ter, p.Arg683Leu, p.Arg1110Gln, and IVS28 + 1G>T were highly recurrent in the cohort of the present study. Bi-allelic variants in *DUOX2*, *TSHR* and *TPO* were identified in 24.76% (26/105) of the patients. Monoallelic variants were identified in 28.57% (30/105) of the patients. Oligogenic variants were identified in 19.05% (20/105) of the patients. The most common variant combinations of oligogenic variants were *DUOX2* and *TG*, and *DUOX2* and *SLC26A4*. In addition, 2 patients harbored tri-allelic and tetra-allelic variants in *DUOX2*, respectively. In conclusion, *DUOX2*, *TG*, *TSHR* and *TPO* variants were the most common genetic defects in patients with CH in the neonatal population of Foshan. Specifically, biallelic *DUOX2* variants were highly prevalent in the cohort. Further, the investigation provided a variant spectrum of CH-related genes and identified novel variants, which may allow for an improved understanding of the underlying genetic etiology of CH and provide evidence for further molecular epidemiological investigations that can guide preventive and therapeutic programs.

Introduction

Congenital hypothyroidism (CH) is a common neonatal endocrine disorder that is characterized by irreversible neurodevelopmental and growth retardation due to insufficient biosynthesis of thyroid hormones. The incidence of CH is estimated to be between 1:2,000 and 1:4,000 (1), based on newborn screening programs for CH that measure thyroid-stimulating hormone (TSH) and/or thyroxine (T4) levels. In China, the incidence of CH is estimated to be 5.77 per 10,000 live births (2). Previous studies have indicated an increase in the diagnosis of CH, particularly in cases of glands *in situ* (GIS) (3,4), which can be attributed to lower cut-off values for TSH during newborn screening (5). However, the etiology of CH remains unclear.

The majority of CH cases (80-85%) are attributed to thyroid dysgenesis (TD), which may manifest as athyreosis, hypoplasia, ectopic thyroid tissue, or a small thyroid gland. The remaining CH cases (15-20%) are attributed to thyroid dyshormonogenesis (DH), which presents with a normally located intact thyroid gland and, in some cases, compensatory goiter (6). Genetic defects in *DUOX2*, *TG*, *TPO*, *SLC26A4*, *SLC5A5*, *DUOXA2* and *IYD* have been associated with inadequate thyroid hormone biosynthesis (7,8). Although TD is typically regarded as a sporadic disease, variants in 5 genes (*GLIS3*, *TSHR*, *NKX2-1*, *PAX8* and *FOXE1*) have been reported as monogenic causes of TD (9). Additionally, genetic factors play a significant role in the etiology of some familial forms of TD (10-12). Furthermore, isolated central CH is associated with genes involved in hypothalamic-pituitary-thyroid axis regulation, including *TRHR*, *TSHB* and *IGSF1* (13).

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Next-generation sequencing (NGS) has enabled the genetic screening of patients with CH and comprehensive analysis of CH-related genes, which may reveal the complex genetic etiology and inheritance patterns of CH. Variants in CH-related genes have been identified in populations of different ethnicities from different regions (14-16). In China, multigenic screening of patients with CH and systematic analysis of genotype-phenotype correlations have been conducted in several provinces (17-19). However, little is known about the variant characteristics of CH-related genes in Foshan, China.

In the present study, an NGS panel containing 30 candidate genes was established for multigenic screening of 105 patients with CH, diagnosed through newborn screening programs in Foshan, China. Variant frequencies and the variant spectrum of CH in the neonatal population of Foshan were evaluated.

Materials and methods

Patients. The present retrospective study included 105 unrelated patients with CH and 138 controls at the Foshan Women and Children Hospital between December 2018 and September 2022 (Foshan, China). The inclusion and exclusion criteria for patients were as follows: Subjects with elevated TSH levels and decreased free thyroxine (FT4) levels who were born to non-consanguineous parents. The control subjects were healthy newborns with no detectable inherited metabolic disorders at newborn screening. Of the 105 patients, 56 were males and 49 were females. The median age of the patient cohort at diagnosis was 16 days, with a range of 6 to 355 days. CH diagnosis was based on TSH levels and FT4 levels in the neonatal screening program. Heel prick samples were collected on filter paper and TSH levels were analyzed by a time-resolved fluorescence-based assay using an Auto TRFIA-4 automatic fluorescence immunoassay analyzer (Guangzhou Fenghua Biotechnology Co., Ltd.; http://www.bio-fenghua. com/index.asp). Patients with high TSH (≥10 μIU/ml) levels were called back for further testing. Subsequently, serum TSH, FT4, free triiodothyronine, T4 and triiodothyronine levels were measured on the Roche Cobas e602 analyzer (Roche Diagnostics GmbH) using electrochemiluminescence immunoassays. Thyroid ultrasonography was performed to assess thyroid morphology whenever possible. The present study was approved (approval no. FSFY-MEC-2021-041) by the Medical Ethics Committee of the Foshan Women and Children Hospital, and written informed consent was obtained from the parents of all patients in accordance with the Declaration of Helsinki.

DNA extraction and sequencing. Genomic DNA was extracted from blood spot cards using Nucleic Acid Isolation or Purification Reagent (cat. nos DR-HS-004; Guangzhou Darui Biotechnology Co., Ltd.) according to the manufacturer's protocols. The NGS panel consisted of 30 candidate genes (*BCHE, DUOX2, EZH2, GLI3, GLIS3, IYD, IGSF1, KAT6B, NEFL, NEFM, NKX2-1, NKX2-5, NSD1, PAX8, PHTF1, POU1F1, SERPINA7, TG, UBR1, SH2B3, SLC26A4, SLC5A5, SECISBP2, TPO, DUOXA2, FOXE1, LHX4, TRHR, TSHB* and *TSHR*). Custom primers were designed to generate 687 amplicons. The target gene exon regions comprised 396 regions, and all exons along with 20 bp of the flanking introns of these regions were amplified by multiplex PCR. The total coverage of the target genes was >98% (Table SI). The Ion AmpliSeq

Library Kit Plus and Ion Xpress Barcode Adapters Kits (cat. nos. A35907 and 4474517, respectively; both from Thermo Fisher Scientific, Inc.) were used to prepare DNA libraries for sequencing. The library was then quantified using the Equalbit 1X dsDNA HS Assay Kit (cat. no. EQ121-01; Vazyme, Biotech Co., Ltd.) and the Qubit Fluorometer 3.0 (Thermo Fisher Scientific, Inc.). Targeted sequencing was performed using a single-end 250 bp sequencing method on the DA8600 sequencer (Guangzhou Darui Biotechnology Co., Ltd.) with the Universal Sequencing Kit (semiconductor sequencing; cat. no. DR-CX-A001; Guangzhou Darui Biotechnology Co., Ltd.).

Bioinformatic analysis and classification of variants. The raw data generated by sequencing were analyzed using Torrent Suite Software (v.4.4.3) (Thermo Fisher Scientific, Inc.). Reads were aligned to the human reference genome (hg19) using the Torrent Mapping Alignment Program (v.4.4.11) (Thermo Fisher Scientific, Inc.). The coverage analysis plugin (v.4.4.2.2) (Thermo Fisher Scientific, Inc.) was used to assess the level of sequence coverage and overall quality of the targeted regions. The Variant Caller plugin (v.4.4.3.3) (Thermo Fisher Scientific, Inc.) was used to evaluate variants, and the called variants were annotated using Ensemble's Variant Effect Predictor (v.102) (grch37.ensembl.org/info/docs/tools/vep) based on the 1000 Genomes Project (http://www.1000genomes. org), dbSNP (http://www.ncbi.nlm.nih.gov/), Exome Aggregation Consortium (http://exac.broadinstitute.org/), ClinVar (http://www.ncbi.nlm.nih.gov/clinvar), Human Gene Mutation Database (http://www.hgmd.cf.ac.uk/), Genome Aggregation Database (http://gnomad.broadinstitute.org/), ESP6500 (http://evs.gs.washington.edu/EVS/), Ensembl (http://plants.ensembl.org/index.html),Refseq (http://www.ncbi. nlm.nih.gov/refseq/rsg), OMIM (https://omim.org/), and UCSC (https://genome.ucsc.edu). Functional predictions of the variants were evaluated using dbNSFP (v.4.1) (https://sites.google. com/site/jpopgen/dbNSFP) to obtain prediction scores based on Sorting Intolerant from Tolerant (http://sift-dna.org), Mutation Taster (http://www.mutationtaster.org/), Mutation Assessor (http://mutationassessor.org/r3/), ClinPred (https://sites.google. com/site/clinpred/), CADD (https://cadd.gs.washington.edu/), Polymorphism Phenotyping-2 (http://genetics.bwh.harvard. edu/pph2/), FATHMM (http://fathmm.biocompute.org. uk/), REVEL (https://sites.google.com/site/revelgenomics/), MetaSVM (http://genomics.usc.edu/members/15-member-det ail/36-coco-dong), PROVEAN (http://provean.jcvi.org/index. php), LRT (http://www.genetics.wustl.edu/jflab/lrt_query. html), GERP (http://mendel.stanford.edu/SidowLab/down-loads/gerp/),andSpliceAI(https://spliceailookup.broadinstitute. org/). The detected variants were classified into 5 categories according to the guidelines of the American College of Medical Genetics and Genomics, namely pathogenic, likely pathogenic, variants of uncertain significance, likely benign, or benign. Each pathogenic criterion was weighted as very strong (PVS1), strong (PS1-4), moderate (PM1-6), or supporting (PP1-5), while each benign criterion was weighted as stand-alone (BA1), strong (BS1-4), or supporting (BP1-6). The criteria were selected based on the evidence observed for the variants and then combined to choose a classification from the five categories (20). Variants classified as likely benign or benign were excluded from the subsequent analysis.

Results

Variant frequency in the study population. In total, 91 variants were identified in 78 of the 105 cases (74.29%). These variants were distributed across 16 genes (*DUOX2*, *TG*, *TPO*, *DUOXA2*, *SLC26A4*, *SLC5A5*, *IYD*, *TSHR*, *GLIS3*, *KAT6B*, *NKX2-5*, *LHX4*, *POU1F1*, *SECISBP2*, *IGSF1* and *TRHR*). The most frequently mutated gene was *DOUX2* (50.55%, 46/91), followed by *TG* (10.99%; 10/91), *TSHR* (8.79%; 8/91) and *TPO* (6.59%; 6/91). Two variants [p.Lys530Ter (*DUOX2*) and p.Gly132Arg (*TSHR*)] were homozygous, while the other variants were heterozygous (Table I). The clinical, biochemical and variant information of the 78 patients is shown in Table SII.

Biallelic variants were identified in 24.76% (26/105) of patients with CH: In *DUOX2* (24 cases), *TSHR* (1 case) and *TPO* (1 case). In addition, monoallelic variants were identified in 28.57% (30/105) of the patients: In *DUOX2* (18 cases), *TSHR* (3 cases), *TG* (2 cases), *GLIS3* (2 cases), *SLC5A5* (1 case), *DUOXA2* (1 case), *IYD* (1 case), *TPO* (1 case) and *KAT6B* (1 case). Oligogenic variants were identified in 19.05% (20/105) of the patients. The most common variant combinations of oligogenic variants were *DUOX2* and *TG* (2 cases), and *DUOX2* and *SLC26A4* (2 cases). In total, 2 patients harbored tri-allelic and tetra-allelic variants in *DUOX2*, respectively. Notably, 71.43% (75/105) of the patients harbored variants in at least one gene involved in thyroid hormone biosynthesis (9) (*DUOX2*, *TG*, *TPO*, *DUOXA2*, *SLC26A4*, *SLC5A5*, *IYD* and *TSHR*) (Table SII).

Analysis of pathogenicity. Of the 91 variants across 16 genes, 16 were novel variants, while 75 variants had been previously reported in literature and databases. A total of 13 of the known variants were classified as pathogenic, including 7 variants in *DUOX2* (p.Arg1110Gln, IVS28+1G>T, p.Arg885Gln, p.Ala1206Thr, p.Arg434Ter, p.Arg701Ter and p.Gln202ThrfsTer99), 3 in *SLC26A4* (IVS7-2A>G, p.Gln696Ter and p.Ser90Leu), 1 in *TPO* (p.Glu757Ter), 1 in *TSHR* (p.Ile654Phe) and 1 in *DUOXA2* (p.Tyr246Ter). A total of 7 known variants were likely pathogenic, including 6 *DUOX2* variants (p.Lys530Ter, p.Ser199TrpfsTer122, p.Gln570Ter, p.Glu160ArgfsTer16, p.Glu879Lys and IVS5-2A>G) and 1 of *DUOXA2* (p.Tyr138Ter). A total of 4 novel variants were likely pathogenic [p.Ile1097LeufsTer24 (*DUOX2*), p.Lys661SerfsTer145 (*GLIS3*), p.Asp1838ThrfsTer14 (*TG*) and p.Glu716Ter (*TPO*)]. Furthermore, 67 variants were classified as variants of uncertain significance (Table I).

The types of variants identified in our cohort are shown in Fig. 1. Most variants were missense variants (69.23%; 63/91), followed by frameshift variants (9.89%; 9/91), stop gained variants (8.79%; 8/91) and intron variants (4.40%; 4/91). Inframe deletion, splice acceptor, protein altering, splice donor and splice region variants were also identified in the present study. In addition, 7 known missense variants in *DUOX2* [p.Arg683Leu (n=10), p.Arg1110Gln (n=8), p.Arg885Gln (n=3), p.Glu879Lys (n=3), p.Thr423Ile (n=3), p.Arg1211His (n=3) and p.Gly437Ala (n=3)] were highly prevalent in the cohort (Table I). A total of 20 patients harbored a stop gained variant in *DUOX2* (p.Lys530Ter). A known splice donor variant (IVS28 + 1G>T) in *DUOX2* was detected in 5 patients (Table SII).

Genotype-phenotype correlation in patients with CH. Of the 78 patients (34 females and 44 males) with variants, 73 patients had normal or goitrous thyroid glands, which may be associated with GIS. Only 1 patient had athyreosis with a monoallelic *GLIS3* mutation (p.Ala908Val). Thyroid morphology was not detected in 4 patients. A total of 7 patients (patients 5,12, 30, 37, 40, 62 and 63) were preterm infants with normal thyroid glands, 5 of whom harbored rare variants in *SLC5A5*, *GLIS3*, *POU1F1*, *DUOXA2*, or *KAT6B*. Furthermore, 10 patients had a family history of thyroid disease, of which 7 (patients 8, 29, 48, 50, 64, 73 and 74) harbored biallelic or monoallelic *DUOX2* variants with eutopic thyroid glands of normal size, and goiter was noted in only 1 case (patient 10) with monoallelic *TG* mutations (p.Asn212Ser). Notably, patient 54 harbored tetra-allelic variants in *DUOX2* (p.Arg683Leu, p.Leu219Ser, p.Gln216delinsLeuSerProGlu and p.Gln216_Leu219del).

Discussion

In the present study, a cohort of 105 patients with CH was comprehensively screened using NGS to analyze the variant frequencies and variant spectrum of CH in the neonatal population of Foshan. Variants in CH-related genes were identified in 74.29% (78/105) of patients. *DUOX2*, *TG*, *TSHR* and *TPO* were the most frequently mutated genes, similar to a previous study in Chinese patients with CH (48).

Variants in *DUOX2* have been reported to be a common cause of CH in patients of Chinese and East Asian ethnicities, often resulting in DH with a normal-sized eutopic or goitrous thyroid gland owing to decreased H_2O_2 production in the thyroid (33,50). Among the 105 patients, 46 different *DUOX2* variants were identified in 61 patients (58.10%; 61/105), reflecting the high prevalence of *DUOX2* variants in patients in the present study, which is consistent with previous studies in Chinese populations (17,19). Moreover, 4 known *DUOX2* variants, p.Lys530Ter, p.Arg683Leu, p.Arg1110Gln, and IVS28 + 1G>T, were highly recurrent in the cohort of the present study. The most common variant detected in the present study was p.Lys530Ter. Consistent with the findings of the present study, Tan *et al* (28) and Fu *et al* (17) reported p.Lys530Ter to be the most common variant in Chinese populations. In the present study, 25.71% (27/105) of the patients had monoallelic variants in *DUOX2*, 29.52% (31/105) had biallelic variants in *DUOX2* and 1.90% (2/105) had tri-allelic variants in *DUOX2* (patients 13 and 69). In addition, 1 patient (patient 54) with tetra-allelic variants in *DUOX2*, whose brother was also diagnosed with CH was also identified. However, additional information regarding patients 13, 54 and 69 has not been collected, which limited the evaluation of their types or inheritance patterns of CH.

TG variants have been reported to affect the synthesis and storage of thyroid hormones, resulting in hypothyroidism with compensatory goiter (51). The variant frequency for *TG* in the cohort of the present study was 9.52% (10/105), which was higher than that reported for Japanese patients (2.82%) (52). In total, 8/10 patients with *TG* variants in the cohort harbored monoallelic heterozygous *TG* variants in combination with other CH-related genes, suggesting that oligogenic involvement may contribute to the genetic etiology of some patients with CH. The *TG* variant p.Arg2585Trp has been reported in both Chinese and Japanese populations (24,53).

Table I. Spectrum of 91 variants in 16 genes.

Gene	Nucleotide change	Amino acid change	Exon/Intron position	dbSNP number	Variant type	Status	Zygosity	Allele frequency (gnomAD)	Evidence of classification	Variant classification	Numbers of patients	(Refs.)
DUOX2	c.1588A>T	p.Lys530 Ter	Exon 14	rs180671269	Stop gained	Known	Het/Hom	0.0092	PVS1_VeryStrong + PM2_Supporting	LP	20	(21)
DUOX2	c.3329G>A	p.Arg1110 Gln	Exon 25	rs368488511	Missense	Known	Het	0.00244645	PM3_VeryStrong + PM2_Supporting + PP1 + PS3_Supporting + PP3_Moderate	P	8	(22)
DUOX2	c.3693+ 1G>T	IVS28+ 1G>T	Intron 28	rs200717240	Splice donor	Known	Het	0.001413	PM3_VeryStrong + PM2_Supporting + PM3_Strong	P	5	(23)
DUOX2	c.2654G>A	p.Arg885 Gln	Exon 20	rs181461079	Missense	Known	Het	0.0011	PM3_Strong + PM2_Supporting + PM5_Moderate + PP3 + PP1 + PS3 Supporting	P	3	(21)
DUOX2	c.596del	p.Ser199 TrpfsTer122	Exon 6	rs766103168	Frame-shift	Known	Het	0.0002	PVS1_VeryStrong + PM2_Supporting	LP	2	(24)
DUOX2	c.3616G>A	p.Ala1206 Thr	Exon 28	rs762588205	Missense	Known	Het	0.0002	PM3_Strong + PM2_Supporting + PS3_Supporting	P	1	(25)
DUOX2	c.1300C>T	p.Arg434 Ter	Exon 12	rs119472026	Stop gained	Known	Het	0.00010873	PVS1_VeryStrong + PM3_VeryStrong + PM2_Supporting	P	1	(26)
DUOX2	c.2101C>T	p.Arg701 Ter	Exon 17	rs201109959	Stop gained	Known	Het	0.00010874	PVS1_VeryStrong + PM3_Strong + PM2_Supporting	P	1	(26)
DUOX2	c.1708C>T	p.Gln570 Ter	Exon 15	rs1332668133	Stop gained	Known	Het	0.0003	PVS1_VeryStrong + PM2_Supporting	LP	1	(27)
DUOX2	c.477del	p.Glu160 ArgfsTer16	Exon 5	rs1480917996	Frame-shift	Known	Het	NA	PVS1_VeryStrong + PM2_Supporting	LP	1	(28)
DUOX2	c.602dup	p.Gln202 ThrfsTer99	Exon 6	rs567500345	Frame shift	Known	Het	0/0.001285	PVS1_VeryStrong + PM3_VeryStrong + PM2_Supporting + PP1	P	1	(29)

Table I. Continued.

Gene	Nucleotide change	Amino acid change	Exon/ Intron position	dbSNP number	Variant type	Status	Zygosity	Allele frequency (gnomAD)	Evidence of classification	Variant classification	Numbers of patients	(Refs.)
DUOX2	c.2635G>A	p.Glu879Lys	Exon 20	rs774556391	Missense	Known	Het	0.00092421	PM3_Strong+PM2_Supporting + PP3_Moderate + PP1 + Supporting + PS3_Supporting	LP	3	(21)
DUOX2	c.2104_2106del	p.Gly702del	Exon 17	rs779340990	Inframe deletion	Known[a]	Het	0.001033	PM2_Supporting + PM4	VUS	2	NA
DUOX2	c.3285_3286del	p.Ile1097LeufsTer24	Exon 25	NA	Frame-shift	Novel	Het	NA	PVS1_VeryStrong + PM2_Supporting	LP	1	NA
DUOX2	c.2048G>T	p.Arg683Leu	Exon 17	rs8028305	Missense	Known	Het	0.00462107	BS1_Strong	VUS	10	(17)
DUOX2	c.1268C>T	p.Thr423Ile	Exon 12	rs201197899	Missense	Known	Het	0.0016	PM2_Supporting	VUS	3	(30)
DUOX2	c.3632G>A	p.Arg1211His	Exon 28	rs141763307	Missense	Known	Het	0.0003262	PP3_Strong + PM2_Supporting	VUS	3	(23)
DUOX2	c.1310G>C	p.Gly437Ala	Exon 12	rs769796932	Missense	Known	Het	0.0017	PP3 + PM2 Supporting	VUS	3	(19)
DUOX2	c.3689C>T	p.Ala1230Val	Exon 28	rs557220354	Missense	Known[a]	Het	0.0002175	PM2_Supporting + BP4g	VUS	2	NA
DUOX2	c.505C>T	p.Arg169Trp	Exon 5	rs201590426	Missense	Known	Het	0.001935	PM2_Supporting + PM5	VUS	2	(31)
DUOX2	c.364C>A	p.Pro122Thr	Exon 5	rs200265605	Missense	Known	Het	0.0004	PM2_Supporting + BP4g	VUS	1	(19)
DUOX2	c.1428C>A	p.Asn476Lys	Exon 13	rs199918362	Missense	Known	Het	0.0053	BS1_Strong + BP4g	VUS	1	(16)
DUOX2	c.4537G>C	p.Gly1513Arg	Exon 34	rs748262140	Missense	Known	Het	NA/ 0.000002052	PP3_Strong + PM2_Supporting	VUS	1	(23)
DUOX2	c.2291G>A	p.Arg764Gln	Exon 18	rs201884203	Missense	Known	Het	0.00010873	PM2_Supporting	VUS	1	(27)
DUOX2	c.4561G>T	p.Gly1521Ter	Exon 34	rs765781255	Stop gained	Known	Het	0.001	PVS1_Moderate + PM2_Supporting	VUS	1	(23)
DUOX2	c.1946C>A	p.Ala649Glu	Exon 17	rs748793969	Missense	Known	Het	5.44E-05	PM3_Strong + PM2_Supporting	VUS	1	(21)
DUOX2	c.1295G>A	p.Arg432His	Exon 12	rs530736554	Missense	Known	Het	0.0006	PM2_Supporting	VUS	1	(23)

Table I. Continued.

Gene	Nucleotide change	Amino acid change	Exon/Intron position	dbSNP number	Variant type	Status	Zygosity	Allele frequency (gnomAD)	Evidence of classification	Variant classification	Numbers of patients	(Refs.)
DUOX2	c.3251G>A	p.Arg1084Gln	Exon 25	rs558919433	Missense	Known	Het	0.0004	PP3_Moderate + PM2_Supporting	VUS	1	(32)
DUOX2	c.4348T>C	p.Tyr1450His	Exon 32	rs753591292	Missense	Known	Het	0.0004	PP3 + PM2_Supporting	VUS	1	(33)
DUOX2	c.3595C>G	p.Leu1199Val	Exon 28	NA	Missense	Novel	Het	NA	PM2_Supporting	VUS	1	NA
DUOX2	c.2335-25T>C	IVS18-25T>C	Intron 18	NA	Intron	Novel	Het	NA	BP7_Supporting + PM2_Supporting	VUS	1	NA
DUOX2	c.1304A>G	p.Asp435Gly	Exon 12	rs772040742	Missense	Known	Het	0.00043492	PP3 + PM2_Supporting	VUS	1	(23)
DUOX2	c.4408C>T	p.Arg1470Trp	Exon 33	rs200785525	Missense	Known	Het	0.0022	PM2_Supporting + PP3_Moderate	VUS	1	(18)
DUOX2	c.1855G>T	p.Val619Leu	Exon 16	rs768447406	Missense	Known[a]	Het	0.0007068	BP4g + PM2_Supporting	VUS	1	NA
DUOX2	c.903G>T	p.Trp301Cys	Exon 8	rs56196384	Missense	Known	Het	0.0003263	PP3 + PM2_Supporting	VUS	1	(17)
DUOX2	c.2412C>G	p.Cys804Trp	Exon 19	NA	Missense	Novel	Het	NA	PP3 + PM2_Supporting	VUS	1	NA
DUOX2	c.655_656delinsTC	p.Leu219Ser	Exon 6	NA	Missense	Novel	Het	NA	PM2_Supporting	VUS	1	NA
DUOX2	c.646_647insTTTCCCCCG	p.Gln216delinsLeuSerProGlu	Exon 6	rs1894400616	Protein altering	Known[a]	Het	NA/0.000016	PM4 + PM2_Supporting	VUS	1	NA
DUOX2	c.647_658del	p.Gln216_Leu219del	Exon 6	NA	Inframe deletion	Novel	Het	NA	PM4 + PM2_Supporting	VUS	1	NA
DUOX2	c.2921G>A	p.Arg974His	Exon 22	rs778216481	Missense	Known	Het	0.0013	PP3 + PM2_Supporting	VUS	1	(19)
DUOX2	c.1127G>A	p.Arg376Gln	Exon 10	rs778729877	Missense	Known[a]	Het	0.00005437	PM5 + PM2_Supporting	VUS	1	NA
DUOX2	c.514-2A>G	IVS5-2A>G	intron 5	NA	Splice acceptor	Known	Het	NA	PVS1_VeryStrong + PM2_Supporting	LP	1	(28)
DUOX2	c.3374A>G	p.Asp1125Gly	Exon 25	NA	Missense	Novel	Het	NA	PP3_Moderate + PM2_Supporting	VUS	1	NA

Table I. Continued.

Gene	Nucleotide change	Amino acid change	Exon/Intron position	dbSNP number	Variant type	Status	Zygosity	Allele frequency (gnomAD)	Evidence of classification	Variant classification	Numbers of patients	(Refs.)
DUOX2	c.4375G>A	p.Asp1459Asn	Exon 32	rs199546504	Missense	Known	Het	0.0005	PM2_Supporting	VUS	1	(27)
DUOX2	c.2894C>T	p.Ser965Leu	Exon 22	rs144153950	Missense	Known	Het	0.0005	PM2_Supporting	VUS	1	(18)
DUOX2	c.1393C>A	p.Pro465Thr	Exon 12	rs774177514	Missense	Known[a]	Het	NA	BP4 + PM2_Supporting	VUS	1	NA
DUOXA2	c.738C>G	p.Tyr246Ter	Exon 5	rs4774518	Stop gained	Known	Het	0.0019	PVS1_Strong + PM3_Strong + PM2_Supporting + PS3_Supporting	P	1	(34)
DUOXA2	c.413dup	p.Tyr138Ter	Exon 4	rs778410503	Frameshift	Known	Het	0.0033	PVS1_VeryStrong + PM2_Supporting	LP	1	(35)
GLIS3	c.1982del	p.Lys661SerfsTer145	Exon 6	NA	Frameshift	Novel	Het	NA	PVS1_VeryStrong + PM2_Supporting	LP	2	NA
GLIS3	c.1843G>A	p.Ala615Thr	Exon 5	rs752946704	Missense	Known[a]	Het	0.000054371	PM2_Supporting	VUS	1	NA
GLIS3	c.2723C>T	p.Ala908Val	Exon 11	rs140101069	Missense	Known	Het	0.004245	BP4 + PM2_Supporting	VUS	1	(36)
IGSF1	c.584G>C	p.Gly195Ala	Exon 5	rs745841814	Missense	Known[a]	Het	0.0003	PM2_Supporting	VUS	1	NA
IYD	c.688-7G>A	IVS4-7G>A	Intron 4	rs1778273239	Splice region	Known[a]	Het	NA/0.000001446	BP4+PM2_Supporting	VUS	1	NA
IYD	c.380C>T	p.Pro127Leu	Exon 3	rs372196319	Missense	Known[a]	Het	0.0001089	PP3_Moderate + PM2_Supporting	VUS	1	NA
KAT6B	c.1025T>C	p.Ile342Thr	Exon 7	rs182392778	Missense	Known[a]	Het	0.0028	BS1_Strong	VUS	2	NA
LHX4	c.970G>A	p.Ala324Thr	Exon 6	rs544059210	Missense	Known	Het	0.00005437	PM2_Supporting	VUS	1	(37)
LHX4	c.1127C>T	p.Thr376Ile	Exon 6	rs1334926032	Missense	Known[a]	Het	NA	PM2_Supporting	VUS	1	NA
NKX2-5	c.773G>C	p.Gly258Ala	Exon 2	NA	Missense	Novel	Het	NA	PP2 + PM2_Supporting	VUS	1	NA
POU1F1	c.744-6C>A	IVS5-6C>A	Intron 5	NA	Intron	Novel	Het	NA	PP3_Moderate + PM2_Supporting	VUS	1	NA
SECISBP2	c.1212+4C>T	IVS8+4C>T	Intron8	NA	Intron	Novel	Het	NA	BP4 + PM2_Supporting	VUS	1	NA
SLC26A4	c.919-2A>G	IVS7-2A>G	Intron 7	rs111033313	Splice acceptor	Known	Het	0.0048	PVS1_VeryStrong + PM3_VeryStrong + PM2_Supporting + PP1 + PS3_Supporting	P	1	(38)

Table I. Continued.

Gene	Nucleotide change	Amino acid change	Exon/Intron position	dbSNP number	Variant type	Status	Zygosity	Allele frequency (gnomAD)	Evidence of classification	Variant classification	Numbers of patients	(Refs.)
SLC26A4	c.2086C>T	p.Gln696Ter	Exon 18	rs752807925	Stop gained	Known	Het	0.0002	PVS1_VeryStrong + PM3_VeryStrong + PM2_Supporting	P	1	(39)
SLC26A4	c.269C>T	p.Ser90Leu	Exon 3	rs370588279	Missense	Known	Het	0.00005437	PM3_VeryStrong + PM1 + PP2 + PM2_Supporting + PP3 + PS3_Supporting	P	1	(40)
SLC26A4	c.-3-46C>T	IVS1-46C>T	Intron 1	NA	Intron	Novel	Het	NA	BP7 + PM2_Supporting	VUS	1	NA
SLC26A4	c.697G>C	p.Val233Leu	Exon 6	rs397516431	Missense	Known	Het	0.0014135	PM3_Strong + PM1 + PP2 + PM2_Supporting + PP3	VUS	1	(41)
SLC5A5	c.1499C>T	p.Pro500Leu	Exon 12	rs531134045	Missense	Known[a]	Het	0.0003	PM2_Supporting	VUS	1	NA
TG	c.5512del	p.Asp1838ThrfsTer14	Exon 29	NA	Frameshift	Novel	Het	NA	PVS1_VeryStrong + PM2_Supporting	LP	1	NA
TG	c.5854C>T	p.Arg1952Trp	Exon 31	rs369705913	Missense	Known[a]	Het	0.00010903	PM2_Supporting	VUS	1	NA
TG	c.3641G>A	p.Arg1214Gln	Exon 17	rs200877580	Missense	Known[a]	Het	0.0002	BP4 + PM2_Supporting	VUS	1	NA
TG	c.635A>G	p.Asn212Ser	Exon 5	rs187737243	Missense	Known[a]	Het	0.0021	BP4	VUS	1	NA
TG	c.1597G>A	p.Gly533Arg	Exon 9	NA	Missense	Novel	Het	NA	PM2_Supporting	VUS	1	NA
TG	c.958C>T	p.Arg320Cys	Exon 8	rs138561283	Missense	Known[a]	Het	0.0008	PM2_Supporting	VUS	1	NA
TG	c.7753C>T	p.Arg2585Trp	Exon 44	rs114211101	Missense	Known	Het	0.00513651	BS1	VUS	1	(18)
TG	c.8205del	p.Gln2736SerfsTer10	Exon 48	rs758002273	Frameshift	Known[a]	Het	0.0014	PVS1_Moderate + PM2_Supporting	VUS	1	NA
TG	c.925A>G	p.Thr309Ala	Exon 8	rs199712883	Missense	Known	Het	0.001	BP4 + PM2_Supporting	VUS	1	(16)
TG	c.3040G>A	p.Asp1014Asn	Exon 12	rs114772213	Missense	Known	Het	0.0005	BP4 + PM2_Supporting	VUS	1	(42)

Table I. Continued.

Gene	Nucleotide change	Amino acid change	Exon/Intron position	dbSNP number	Variant type	Status	Zygosity	Allele frequency (gnomAD)	Evidence of classification	Variant classification	Numbers of patients	(Refs.)
TPO	c.2268dup	p.Glu757Ter	Exon 13	rs770781635	Frameshift	Known	Het	0.0016	PVS1_VeryStrong + PM3_VeryStrong + PM2_Supporting	P	1	(43)
TPO	c.2146G>T	p.Glu716Ter	Exon 12	NA	Stop gained	Novel	Het	NA	PVS1_VeryStrong + PM2_Supporing	LP	1	NA
TPO	c.2017G>A	p.Glu673Lys	Exon 12	rs201193196	Missense	Known	Het	0.0007	PP3 + PM2_Supporting	VUS	1	(44)
TPO	c.2603C>T	p.Thr868Met	Exon 15	rs201576336	Missense	Known[a]	Het	0.0009	PM2_Supporting	VUS	1	NA
TPO	c.2536C>T	p.Arg846Trp	Exon 15	rs28913014	Missense	Known	Het	0.0017	BS1 + BS2_Supporting	VUS	1	(45)
TPO	c.1367G>A	p.Arg456Lys	Exon 9	rs1329337261	Missense	Known[a]	Het	0	PM2_Supporting	VUS	1	NA
TRHR	c.504T>G	p.Ile168Met	Exon 2	rs13306060	Missense	Known	Het	0.0024	BP4+PM2_Supporting	VUS	1	(46)
TSHR	c.2272G>A	p.Glu758Lys	Exon 10	rs746522401	Missense	Known	Het	0.0003262	PM2_Supporting	VUS	1	(32)
TSHR	c.740T>C	p.Val247Ala	Exon 2	NA	Missense	Novel	Het	NA	BP4 + PM2_Supporting	VUS	1	NA
TSHR	c.394G>C	p.Gly132Arg	Exon 5	rs760874290	Missense	Known	Hom	0.00054413	PS4+PM2_Supporting + PP3_Supporting	VUS	1	(47)
TSHR	c.823G>A	p.Ala275Thr	Exon 9	rs180762551	Missense	Known	Het	0.0003262	PP3 + PM2_Supporting	VUS	1	(48)
TSHR	c.915T>A	p.Ser305Arg	Exon 10	rs142122217	Missense	Known	Het	0.0033	NA	VUS	1	(48)
TSHR	c.1492G>A	p.Gly498Ser	Exon 10	rs1376842882	Missense	Known	Het	NA	PM2_Supporting + PP3_Moderate + PM1	VUS	1	(49)
TSHR	c.694G>C	p.Asp232His	Exon 9	rs752791414	Missense	Known[a]	Het	0.00005437	PP3_Moderate + PM2_Supporting	VUS	1	NA
TSHR	c.1960A>T	p.Ile654Phe	Exon 10	rs767239688	Missense	Known[a]	Het	0.0002	PP3_Strong + PS4 + PM2_Supporting	P	1	NA

[a]Variants recorded in database of dbSNP or gnomAD. NA, data not available; Het, heterozygous; Hom, homozygous; P, pathogenic; LP, likely pathogenic; VUS, variants of uncertain significance.
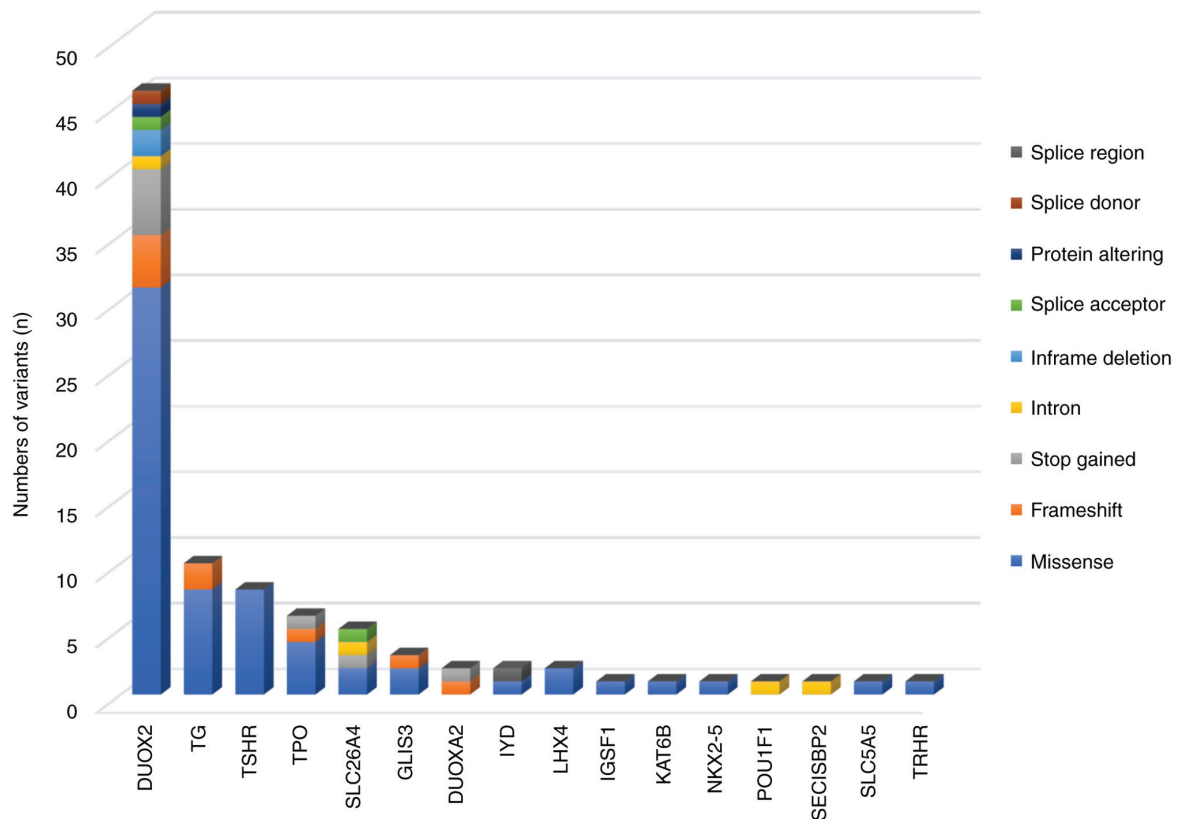
Figure 1. Distribution of 91 variants and their variant type across 16 genes. Of the 91 variants, 46 were detected in *DUOX2*, 10 in *TG*, 8 in *TSHR*, 6 in *TPO*, 5 in *SLC26A4*, 3 in *GLIS3*, 2 each in *DUOXA2*, *IYD* and *LHX4* and 1 each in *IGSF1*, *KAT6B*, *NKX2-5*, *POU1F1*, *SECISBP2*, *SLC5A5* and *TRHR*. Missense, frameshift, stop gained, intron, in-frame deletion, splice acceptor, splice donor, splice region and protein-altering variants were identified in the present study.

Inactivating variants in *TSHR* cause TSH resistance, which negatively affects thyroid growth, and stimulates thyroid hormone synthesis and release (7). Monoallelic *TSHR* variants were identified in 6 patients (5.71%; 6/105), and *DUOX2* or *TG* variants were found along with *TSHR* variants in 3/6 patients with *TSHR* variants. Biallelic variants in *TSHR* were identified in 1 patient whose sister also had CH. In contrast to Chinese populations, *TSHR* variants are the most common genetic defects in patients with CH (10.9%) in Saudi Arabia (54). In the present study, *TSHR* variants-p.Glu758Lys, p.Ala275Thr and p.Ser305Arg-were reported in the Chinese population (32,48,55). p.Gly132Arg has been frequently reported in patients with CH of Chinese (50), Japanese (47) and Korean (14) ethnicities. In total, 1 patient in the cohort has a homozygous *TSHR* variant p.Gly132Arg. p.Gly498Ser has been previously reported in the Japanese population, and its low expression is likely to affect the functions of the TSH receptor (49).

TPO plays an important role in thyroid hormone biosynthesis and variants in *TPO* have been reported to be highly prevalent in patients with DH-associated CH of Caucasian (56) and Malaysian-Chinese (57) ethnicities. In the cohort of the present study, the variant rate for *TPO* was ~4.76% (5/105), which was lower than that reported in other populations. The stop gained variant p.Glu757Ter identified in the cohort of the present study has been reported as a common cause of CH in Taiwanese (43).

In addition, no definite pathogenic or likely pathogenic variants were identified in *IYD*, *SLC5A5*, *LHX4*, *IGSF1*, *KAT6B*, *NKX2-5*, *POU1F1*, *SECISBP2*, or *TRHR* in the cohort. Moreover, variants in these genes appear to be rare: Only 1 or 2 variants were identified for each of these genes, and are usually associated with variants in other genes, especially *DUOX2* or *TG*.

A previous study reported that oligogenic variants are common in sporadic CH (16), suggesting that a combination of rare variations in CH-related genes may underlie the complex genetic etiology of CH. In the present study, oligogenic variants were detected in 19.05% (20/105) of the patients, and the combination of *DUOX2*, *TG*, *TPO* and *TSHR* variants was noted frequently. There is some evidence that suggests that patients with tri-allelic variants have permanent CH (17). However, one of the limitations of the present study is that the clinical phenotypes of all patients with CH in the cohort of the present study were not clearly elucidated; therefore, the association between the function of oligogenic variants and the hypothyroid phenotype remains unclear.

In conclusion, *DUOX2*, *TG*, *TSHR* and *TPO* variants were the most common genetic defects in patients with CH in the neonatal population of Foshan. Specifically, biallelic *DUOX2* variants were highly prevalent in the study population. Based on the findings of the present study, the authors suggest that oligogenic variants in CH-related genes may contribute to the complex genetic etiology of CH. Further, the present investigation provides a detailed variant spectrum of CH-related genes and identifies novel variants, which may allow for an improved understanding of the underlying genetic etiology of CH and provide evidence for further molecular epidemiological investigations that can guide preventive and therapeutic programs in Foshan, China.

Availability of data and materials

The data generated in the present study may be found in the Genome Sequence Archive for Human under accession number HRA008474 or at the following URL: https://ngdc.cncb.ac.cn/gsa-human/s/U36l4c36.

Authors' contributions

WC collected the patients' blood samples. WC, SW and WY performed the experiments. XH, QS and JT analyzed and interpreted the data. SW, XY and XH drafted and wrote the manuscript. XH, XY and SW participated in discussing and revising the manuscript. XS and XY conceived and designed the study. XS, XH and JT contributed to overall senior mentorship and guidance and support to the project. XH and SW confirm the authenticity of all the raw data. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

The present study was approved by the Medical Ethics Committee of the Foshan Women and Children Hospital (Foshan, China) on 12 March 2021 (approval no. FSFY-MEC-2021-041), and the renewal date of the ethics approval was 22 May 2025. Written informed consent was obtained from the parents/guardians of all patients and controls in accordance with the Declaration of Helsinki.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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