RESEARCH

DUOX2 variants are a frequent cause of congenital primary hypothyroidism in Thai patients

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Abstract

Objective: To identify the genetic etiologies of congenital primary hypothyroidism (CH) in Thai patients.

Design and methods: CH patients were enrolled. Clinical characteristics including age, signs and symptoms of CH, pedigree, family history, screened thyroid-stimulating hormone results, thyroid function tests, thyroid imaging, clinical course and treatment of CH were collected. Clinical exome sequencing by next-generation sequencing was performed. In-house gene list which covered 62 potential candidate genes related to CH and thyroid disorders was developed for targeted sequencing. Sanger sequencing was performed to validate the candidate variants. Thyroid function tests were determined in the heterozygous parents who carried the same *DUOX2* or *DUOXA2* variants as their offsprings.

Results: There were 118 patients (63 males) included. Mean (SD) age at enrollment was 12.4 (7.9) years. Forty-five of 118 patients (38%) had disease-causing variants. Of 45 variants, 7 genes were involved (*DUOX2*, *DUOXA2*, *TG*, *TPO*, *SLC5A5*, *PAX8* and *TSHR*). *DUOX2*, a gene causing thyroid dyshormonogenesis, was the most common defective gene (25/45, 56%). The most common *DUOX2* variant found in this study was c.1588A>T. *TG* and *TPO* variants were less common. Fourteen novel variants were found. Thyroid function tests of most parents with heterozygous state of *DUOX2* and *DUOXA2* variants were normal.

Conclusions: DUOX2 variants were most common among Thai CH patients, while *TG* and *TPO* variants were less common. The c.1588A>T in *DUOX2* gene was highly frequent in this population.

Key Words

- congenital hypothyroidism
- next generation sequencing
- DUOX2
- thyroid dyshormonogenesis
- thyroid dysgenesis
- goiter

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Introduction

Congenital primary hypothyroidism (CH) is classified into thyroid dysgenesis (TD) and thyroid dyshormonogenesis (TDH) (1). TDH has increasingly been reported while the incidence of TD has remained stable (2, 3). Genetic studies have provided more information on the causes of CH (2, 4, 5, 6). To date, more than 20 disease-causing genes have

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been reported to be linked with the pathogenesis of CH (1, 7, 8, 9, 10). TD is defined as abnormal thyroid gland development including ectopic gland, hypoplasia and athyreosis. Genetic etiologies of TD include *TSHR*, *NKX2-1*, *FOXE1*, *PAX8*, *NKX2-5*, *GLIS3*, *JAG1*, *TBX1*, *NTN1* and *CDCA8* variants (1, 11). TDH is characterized by thyroid hormone biosynthetic defect. Genetic defects involved in the steps of thyroid hormone synthesis pathway include *SLC5A5*, *SLC26A4*, *DUOX1*, *DUOX2*, *DUOXA1*, *DUOXA2*, *TPO*, *TG*, *IYD* and *GNAS* genes (1, 11).

Identifying genetic causes of CH has several advantages for patients. Genetic diagnosis provides a risk estimation of thyroidal and extrathyroidal defects in affected patients and families and helps in predicting long-term prognosis in affected individuals (1). Owing to the fact that CH is a genetically heterogeneous disorder which is caused by variants of various genes, traditional sequencing of candidate genes of CH demonstrated pathogenic variants in only approximately 10% of the reported cases (12). Currently, next-generation sequencing (NGS) analysis has been reported to provide an efficient, cost-effective and multigenic screening tool to establish the genetic causes of CH with the diagnostic yield of 46–59% (4, 5, 6).

The incidence of CH has been increasing worldwide. Previous studies reported varied incidences of CH depending on race and ethnicity (13, 14, 15). The CH incidence was reported at 1:1200–2380 in Asians and 1:3533–11,000 in Caucasians (13, 14). TDH was found to be more frequent than TD in patients from China, Iran and United Arab Emirates (2, 16, 17). Genetic analysis revealed that TDH was more frequently associated with *DUOX2* variants in patients of Asian origin, including Japan, Korea and China, and with *TG* and *TPO* variants in patients from United Kingdom and Finland (2, 5, 6, 18, 19). This study aimed to investigate the clinical and molecular characteristics of Thai patients with CH.

Materials and methods

Patients

All enrolled CH patients were regularly treated at the Departments of Pediatrics and Medicine, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok, Thailand. CH was diagnosed based on the findings of elevated serum thyroid-stimulating hormone (TSH) and low free thyroxine (FT_4) concentrations on either confirmatory test for positive newborn screening (NBS) or thyroid function tests for other signs and symptoms suggesting CH. Patients with transient CH secondary to maternal conditions, sick euthyroid syndrome and obvious syndromic features were excluded.

Provisional clinical diagnoses of TDH and TD were made in patients who had clinical features, and possibly thyroid scintigraphic or ultrasonographic findings suggestive of the particular diagnoses. Patients with goiter, or normal eutopic or enlarged thyroid gland on the thyroid imaging were classified as having TDH while patients who had absent or small or ectopic thyroid gland on thyroid imaging were considered to have TD. Patients who were not compatible with the two groups were classified as having undetermined cause. Patients with persistently high TSH after levothyroxine (LT_4) discontinuation after 3 years of age were diagnosed as having permanent CH. 'Transient' CH was diagnosed based on having normal thyroid function test results following discontinuation of LT_4 therapy after 3 years of age and thereafter.

The study was approved by the Ethics Committee on Human Research of the Faculty of Medicine Ramathibodi Hospital, Mahidol University (MURA 2018/844, dated 6 December 2018). The study conformed with the Declaration of Helsinki. Written informed consent was obtained from the patients or their legal guardians.

Clinical data collection

Clinical characteristics including age, signs and symptoms of CH, pedigree, family history of CH, TSH screening results, thyroid function tests, thyroid imagings, clinical course and treatment of CH were collected.

DNA extraction and targeted sequencing of candidate genes

Genomic DNA was extracted from peripheral blood using the QuickGene DNA Whole Blood Kit L (Kurabo, Japan). DNA of the patients was submitted for clinical exome sequencing (CES). CES by NGS was performed by Illumina MiSeq® system (Illumina, USA) using the TruSight One Sequencing Panel®. The TruSight One Sequencing Panel® focused on 4811 known disease-causing genes that have been reported to be associated with human diseases. Sequences were aligned with the human reference genome version hg19. Thyroid disorder gene list including genes related to CH, secondary hypothyroidism, thyroid hormone resistance, thyroid hormone metabolism defects and thyroid test abnormalities without thyroid pathology (such as *ALB* and *SERPINA7*) was developed in-house. It covered 62 potential candidate genes (Table 1) which are

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Table 1 Sixty-two genes that are related to thyroid disorders and covered by the panel used in this study.

Classification	Genes	OMIM number	Phenotypes	Inheritance
Thyroid dysgenesis	*NKX2-1*	600635	Choreoathetosis, hypothyroidism and neonatal respiratory distress	AD
	FOXE1	602617	Bamforth-Lazarus syndrome	AR
	PAX8	167415	Thyroid dysgenesis or hypoplasia	AD
	NKX2-5	600584	Congenital nongoitrous hypothyroidism	AD
	GLIS3	610192	Neonatal diabetes mellitus with congenital hypothyroidism	AR
	TSHR	603372	Congenital nongoitrous hypothyroidism	AR, AD
	JAG1	601920	Alagille syndrome	AD
	TBX1	602054	DiGeorge syndrome	AD
Thyroid dyshormonogenesis	*SLC5A5*	601843	Thyroid dyshormonogenesis	AR
	TPO	606765	Thyroid dyshormonogenesis	AR
	SLC26A4	605646	Pendred syndrome	AR
	TG	188450	Thyroid dyshormonogenesis	AR
	IYD	612025	Thyroid dyshormonogenesis	AR
	DUOX2	606759	Thyroid dyshormonogenesis	AR, AD
	DUOXA2	612772	Thyroid dyshormonogenesis	AR, AD
	GNAS	139320	Pseudohypoparathyroidism	AD
Central hypothyroidism	*TSHB*	188540	Congenital nongoitrous hypothyroidism	AR
	TRHR	188545	Congenital nongoitrous hypothyroidism	AR
	TBL1X	300196	Congenital nongoitrous hypothyroidism	XLR
	HESX1	601802	Combined pituitary hormone deficiencies	AD, AR
	LHX3	600577	Combined pituitary hormone deficiencies	AR
	LHX4	602146	Combined pituitary hormone deficiencies	AD
	SOX3	313430	Panhypopituitarism	XLR
	OTX2	600037	Combined pituitary hormone deficiencies	AD
	POU1F1	173110	Combined pituitary hormone deficiencies	AD, AR
	PROP1	601538	Combined pituitary hormone deficiencies	AR
	IRS4	300904	Congenital nongoitrous hypothyroidism	XLR
Thyroid hormone resistance and abnormal thyroid hormone metabolism	*THRB*	190160	Thyroid hormone resistance	AD, AR
	THRA	190120	Congenital nongoitrous hypothyroidism	AD
	SLC16A2	300095	Allan-Herndon-Dudley syndrome	XLR
	SECISBP2	607693	Abnormal thyroid hormone metabolism	AR
Syndromes or transcription factors which may be associated with congenital hypothyroidism	*SALL1*	602218	Townes-Brocks syndrome	AD
	UBR1	605981	Johanson-Blizzard syndrome	AR
	DYRK1A	600855	Mental retardation	AD
	ELN	130160	Supravalvular aortic stenosis	AD
	KDM6A	300128	Kabuki syndrome	XLD
	KMT2D	602113	Kabuki syndrome	AD
	KAT6B	605880	Genitopatellar syndrome and Say-Barber-Biesecker-Young-Simpson syndrome	AD
	ALB	103600	Dysalbuminemic hyperthyroxinemia	AD
	ALMS1	606844	Alstrom syndrome	AR
	DIO1	147892	Asymptomatic hyperthyroxinemia	AD
	DIO2	601413	Asymptomatic hyperthyroxinemia	ND
	FGF8	600483	Hypogonadotropic hypogonadism with or without anosmia	AD
	HHEX	604420	Thyroid dysgenesis	ND
	NKX2-3	606727	Thyroid dysgenesis	ND
	NKX2-6	611770	Conotruncal heart malformations and persistent truncus arteriosus	AR
	PTH1R	168468	Pseudohypoparathyroidism	ND
	PTRH2	608625	Infantile-onset multisystem neurologic, endocrine, and pancreatic disease	AR
	RYR2	180902	Hyperemesis gravidarum	ND
	SERPINA7	314200	Thyroxine binding globulin deficiency	XLR
	SLC30A10	611146	Hypermanganesemia with dystonia	AR
	SLCO1C1 (OATP1C1)	613389	Thyroid hormone transporter deficiency	AR

(Continued)

Table 1 (Continued)

Classification	Genes	OMIM number	Phenotypes	Inheritance
	TTR	176300	Dystransthyretinemic hyperthyroxinemia	AD
	MC2R	607397	Glucocorticoid deficiency due to ACTH unresponsiveness	AR
	MRAP	609196	Glucocorticoid deficiency	AR
	PDE4D	600129	Acrodysostosis with or without hormone resistance	AD
	PRKAR1A	188830	Acrodysostosis with or without hormone resistance	AD
	TBC1D24	613577	Deafness, onychodystrophy, osteodystrophy, mental retardation and seizures (DOORS) syndrome	AR
	TRAPPC9	611966	Mental retardation	AR
	TXNRD2	606448	Glucocorticoid deficiency	AR
	FOXI1	601093	Enlarged vestibular aqueduct	AR
	KCNJ10	602208	Enlarged vestibular aqueduct	AR

ACTH, adrenocorticotropic hormone; AD, autosomal dominant; AR, autosomal recessive; OMIM, online Mendelian inheritance in men; ND, no data; XLD, X-linked dominant; XLR, X-linked recessive.

known to be related to thyroid disorders according to the previous reports (7, 8, 9, 10). Some genes related to syndromic CH were included to detect genetic variants in patients who might not have recognizable features. Of the 62 genes, there were 16 genes that are related to TD and TDH.

The variant annotation was performed with VarSeq® Software version 2.1.1 (Golden Helix, USA). Candidate variants were filtered based on in-house developed thyroid disorder gene list and minor allele frequency (MAF) of less than 0.05 across the online databases (e.g. gnomAD, 1000 Genomes, ExAC, dbSNP and ClinVar) and in-house Thai database (455 persons). Using the American College of Medical Genetics and Genomics (ACMG) 2015 variant classification guidelines together with Varsome® software (Saphetor, Switzerland), the clinical interpretation of selected variants was determined (20, 21). Computational and prediction data using *in silico* tools were done as one of the ACMG criteria. Variants that were classified as pathogenic or likely pathogenic were considered to be definite causes of CH in the patients. Variants that did not meet the criteria of pathogenic, likely pathogenic, benign or likely benign, would be classified as variant of uncertain significance (VUS). Sanger sequencing was performed to validate the candidate variants in all patients and their parents. In index cases who had siblings with CH, their CH siblings were analyzed for the same variants by Sanger sequencing. Thyroid function tests including FT_4, TSH and thyroglobulin (Tg) concentrations were determined in the heterozygous parents who carried the same *DUOX2* or *DUOXA2* variants as their offsprings. Genotype and phenotype correlation of CH was analyzed.

Statistical analysis

Data were analyzed using SPSS version 22.0 (IBM Corp). Normally and non-normally distributed data were expressed as mean and S.D., and median and interquartile range (IQR), respectively. Mann–Whitney U test was used for comparison between two groups of non-normally distributed data. A *P*-value of less than 0.05 was considered statistically significant.

Results

A total of 120 Thai patients with CH were enrolled. Two patients with syndromic features were excluded. Therefore, 118 patients from 109 families were included in the analysis. Eighteen patients were siblings in 9 families. There was no history of consanguinity. There were 55 females and 63 males. Mean (S.D.) age at enrollment was 12.4 (7.9) years. Of the 118 patients, 41 (35%), 22 (19%) and 55 (46%) patients were clinically classified as having TDH, TD and undetermined cause, respectively. Ninety-one patients (77%) were identified through positive NBS. The remaining 27 patients presented with hypothyroid-related symptoms (21 patients), ectopic thyroid gland (5 patients) and non-autoimmune thyroid goiter (1 patient). There were 92 and 11 patients with permanent and transient CH, respectively. The remaining 15 patients were less than 3 years of age at the time of enrollment, therefore their permanence awaited to be determined.

CES analysis revealed seven CH-causing genes in 39 out of 109 families (45 out of 118 patients, 38%). Thirty-six out of 45 patients (80%) had variants in the

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genes related to TDH, including *DUOX2* ($n = 25$), *DUOXA2* ($n = 6$), *TG* ($n = 2$), *TPO* ($n = 2$) and *SLC5A5* ($n = 1$); and the remaining 9 patients (20%) had variants in the genes related to TD, including *TSHR* ($n = 5$) and *PAX8* ($n = 4$). There were 14 novel pathogenic variants, including 4 *DUOX2* variants, 2 *DUOXA2* variants, 2 *TG* variants, 1 *SLC5A5* variant, 3 *PAX8* variants and 2 *TSHR* variants (Table 2). There were no pathogenic or likely pathogenic variants in *SLC26A4*, *IYD*, *GNAS*, *NKX2-1*, *FOXE1*, *NKX2-5*, *GLIS3*, *TBX1* and *JAG1* genes. VUS were demonstrated in 8 additional patients among the 118 patients (7%). Among these 8 patients, there were 2 patients who had heterozygous VUS; one had *DUOX2* variant (c.2830G>A) and the other had *DUOXA2* variant (c.122T>C) which might be responsible for their CH phenotype. VUS were not included in the reported positive variants.

Clinical characteristics and details of patients with genetic variants are summarized in Table 3. All pathogenic and likely pathogenic variants are shown in Table 2.

Variants of genes related to TDH

DUOX2 variants were the most frequent cause of TDH. Twenty-two different *DUOX2* variants were identified in 25 patients (23 families). Eighteen out of 25 patients (72%) carried either compound heterozygous or homozygous variants; and the remaining 7 patients (28%) had heterozygous variants. The most common pathogenic *DUOX2* variant was c.1588A>T, in 10 alleles in 9 patients. While this variant is rare in overall population with MAF of 0.0007 from gnomAD database, it is relatively common in Thai population with MAF of approximately 0.01 in 455 ethnic-matched normal control subjects from our in-house Thai database. Four different *DUOXA2* variants were identified in 6 patients (5 families), of which three of them had either compound heterozygous or homozygous variants; and the other three had heterozygous variants. The most common *DUOXA2* variant was c.738C>G, in 5 alleles in 4 patients. Five patients with *DUOX2* variants and 2 patients with *DUOXA2* variants had transient CH and 16 patients with *DUOX2* variants and 3 patients with *DUOXA2* variants had permanent CH. The remaining 4 patients with *DUOX2* variants and 1 patient with *DUOXA2* variant were less than 3 years of age at the time of enrollment, so their permanence awaited to be determined. Hypothyroidism in 27 out of 31 patients (87%) with *DUOX2* and *DUOXA2* variants was detected by NBS while 3 patients had negative NBS results and prolonged jaundice was the presentation of hypothyroidism. The remaining 1 patient who had *DUOXA2* variant presented with enlargement of an ectopic thyroid gland at 5 years of age.

SLC5A5 variant was identified in 1 patient. At 12 years of age following LT_4 therapy discontinuation, his thyroid scintigraphy showed no radiotracer uptake but ultrasonography showed normal thyroid gland. *TPO* variants were detected in 2 patients from the same family. The older brother presented with short stature and diffuse goiter at 8.6 years of age and his sister presented with short stature and multinodular goiter at 6.7 years of age. Additionally, *TG* variants were found in 2 patients.

Variants of genes related to TD

The majority of variants of the genes related to TD were found in *TSHR* gene. Four *TSHR* variants in 5 patients were detected. Of these 5 patients, 4 had either homozygous or compound heterozygous variants and one patient with subclinical hypothyroidism had heterozygous variant. Four patients with *PAX8* variants had varied thyroid phenotypes, including athyreosis, hypoplasia and gland in situ, but absent uptake on thyroid scintigraphy. Two patients presented with short stature during childhood and adolescence.

Genotype-phenotype analysis of patients with *DUOX2* variants

Among 18 patients with biallelic *DUOX2* variants, 11 (61%) had permanent CH, 3 (17%) had transient CH and the remaining 4 (22%) were under 3 years of age, whose permanence awaited to be determined. Out of 7 patients with monoallelic *DUOX2* variants, 5 had permanent CH and 2 had transient CH. Median (IQR) serum TSH and FT_4 concentrations at diagnosis of patients with monoallelic and biallelic variants were not statistically different [TSH: 50.0 (17.7, 100.0) and 50.0 (39.5, 100.0) mU/L, p=0.604; FT_4: 0.9 (0.4, 1.3) and 0.6 (0.4, 0.9) ng/dL, p= 0.482, respectively]. There was no evidence of genotype-phenotype correlation.

Segregation analysis of patients with *DUOX2* and *DUOXA2* variants

Serum FT_4, TSH and Tg concentrations were determined in 29 heterozygous parents from 17 families of patients who carried variants of the *DUOX2* and *DUOXA2* genes (Fig. 1 and Table 4). Regarding patients with compound heterozygous and homozygous variants in the *DUOX2* and

Table 2 Details of pathogenic and likely pathogenic variants of seven genes identified in the study.

Genes	Nucleotide position	Amino acid position	Mutation types	SIFT	Polyphen-2	Allele frequency gnomAD	Thai allele frequency (*n*= 455)	Number of alleles	Status (accession number)	RS number
DUOX2 (NM_014080.4)	c.1588A>T	p.Lys530Ter	Nonsense	NA	NA	0.000675966	0.00989	10	Reported	rs180671269
	c.2654G>A	p.Arg885Gln	Missense	0.006 Deleterious	0.999 Damaging	0.000115324	0.0010989	4	Reported	rs181461079
	c.2048G>T	p.Arg683Leu	Missense	0.002 Deleterious	1.000 Damaging	0.000342275	–	3	Reported	rs8028305
	c.2104_2106delGGA	p.Gly702del	In-frame deletion	NA	NA	0.000075580	0.0010989	3	Reported	rs779340990
	c.2654G>T	p.Arg885Leu	Missense	0.003 Deleterious	0.999 Damaging	0.000405621	–	3	Reported	rs181461079
	c.4027C>T	p.Leu1343Phe	Missense	0.054 Tolerated	0.831 Damaging	0.000592662	0.0054945	3	Reported	rs147945181
	c.1304A>G	p.Asp435Gly	Missense	0.000 Deleterious	1.000 Damaging	0.000031812	–	2	Reported	rs772040742
	c.1310G>C	p.Gly437Ala	Missense	0.000 Deleterious	1.000 Damaging	0.000123275	0.0010989	2	Novel (SCV001250672)	rs769796932
	c.2101C>T	p.Arg701Ter	Nonsense	NA	NA	0.000031826	0.0032967	2	Reported	rs201109959
	c.3693+1G>T	–	Splice site	NA	NA	0.000103437	0.0032967	2	Reported	rs200717240
	c.989T>G	p.Val330Gly	Missense	0.226 Tolerated[a]	0.016 Benign[a]	–	–	1	Novel (SCV001250732)	–
	c.1232G>A	p.Arg411Lys	Missense	0.033 Deleterious	0.372 Benign	0.000059650	–	1	Reported	rs764353021
	c.1295G>A	p.Arg432His	Missense	0.038 Deleterious	0.933 Damaging	0.000067603	–	1	Reported	rs530736554
	c.2635G>A	p.Glu879Lys	Missense	0.000 Deleterious	1.000 Damaging	0.000075555	0.0010989	1	Reported	rs774556391
	c.2895_2898delGTTC	p.Phe966Serfs*29	Frameshift	NA	NA	0.00293655	–	1	Reported	rs530719719
	c.3115C>T	p.Arg1039Trp	Missense	0.000 Deleterious	1.000 Damaging	0.000011936	–	1	Novel (SCV001245530)	rs752176935
	c.3329G>A	p.Arg1110Gln	Missense	0.003 Deleterious	0.994 Damaging	0.000194847	0.0010989	1	Reported	rs368488511
	c.3340delC	p.Leu1114Serfs*56	Frameshift	NA	NA	0.000015905	0.0010989	1	Reported	rs748194265
	c.3478_3480delCTG	p.Leu1160del	In-frame deletion	NA	NA	0.000027845	–	1	Reported	rs758318135
	c.3631C>T	p.Arg1211Cys	Missense	0.000 Deleterious	1.000 Damaging	0.000043753	–	1	Reported	rs374410986
	c.4080G>T	p.Lys1360Asn	Missense	0.068 Tolerated[b]	0.379 Benign[b]	0.000003992	–	1	Novel (SCV001250737)	rs374891282
	c.4408C>T	p.Arg1470Trp	Missense	0.000 Deleterious	1.000 Damaging	0.000159584	0.0010989	1	Reported	rs200785525

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DUOXA2 (NM_207581.3)	c.738C>G	p.Tyr246Ter	Nonsense	NA	NA	0.000143084	0.0021978	5	Reported	rs4774518
	c.604G>A	p.Ala202Thr	Missense	0.016 Deleterious	0.643 Possibly damaging	0.000016892	–	2	Novel (SCV001250673)	rs770148072
	c.232G>A	p.Val78Met	Missense	0.076 Tolerated	1.000 Damaging	0.000044113	–	1	Reported	rs746132852
	c.501C>A	p.Cys167Ter	Nonsense	NA	NA	0.000004014	–	1	Novel (SCV001250908)	rs781126484
TG (NM_003235.4)	c.48G>A	p.Trp16Ter	Nonsense	NA	NA	0.000004367	–	1	Novel (SCV001250735)	rs780846892
	c.274+2T>G	–	Splice site	NA	NA	0.000003991	0.0010989	1	Reported	rs1398373161
	c.1348delT	p.Ser450Profs*29	Frameshift	NA	NA	0.000055760	–	1	Reported	rs776553164
	c.6791G>A	p.Cys2264Tyr	Missense	0.001 Deleterious	1.000 Damaging	0.000011931	–	1	Novel (SCV001250736)	rs1229345000
TPO (NM_000547.5)	c.670_672delGAC	p.Asp224del	In-frame deletion	NA	NA	0.000059679	–	2	Reported	rs772164623
	c.2422delT	p.Cys808Alafs*24	Frameshift	NA	NA	0.000083532	–	2	Reported	rs763662774
SLC5A5 (NM_000453.2)	c.794A>G	p.Gln265Arg	Missense	0.008 Deleterious	0.999 Damaging	–	–	2	Novel (SCV001245529)	–
PAX8 (NM_003466.3)	c.92G>A	p.Arg31His	Missense	0.000 Deleterious	1.000 Damaging	–	–	1	Reported	rs104893657
	c.203C>T	p.Thr68Ile	Missense	0.000 Deleterious	1.000 Damaging	–	–	1	Novel (SCV001245528)	–
	c.236C>T	p.Ser79Phe	Missense	0.000 Deleterious	1.000 Damaging	–	–	1	Novel (SCV001250734)	–
	c.457_458delCT	p.Leu153Glufs*47	Frameshift	NA	NA	–	–	1	Novel (SCV001250738)	–
TSHR (NM_000369.2)	c.1960A>T	p.Ile654Phe	Missense	0.000 Deleterious	1.000 Damaging	0.000011929	0.0021978	4	Novel (SCV001250733)	rs767239688
	c.545+5G>T	–	Splice site	NA	NA	–	–	2	Novel (SCV001250739)	–
	c.1825C>T	p.Arg609Ter	Nonsense	NA	NA	0.000003978	–	2	Reported	rs763679435
	c.1349G>A	p.Arg450His	Missense	0.000 Deleterious	1.000 Damaging	0.000234637	0.0021978	1	Reported	rs189261858

–Absent in database; [a]c.989T>G variant was predicted to be probably deleterious (0.960) by Mutation Taster; [b]c.4080G>T variant was predicted to be probably deleterious (0.999) by Mutation Taster. gnomAD, Genome Aggregation Database (version 2.1.1); NA, not available; Polyphen-2, Polymorphism Phenotypic version 2 (used to predict the effects of missense mutations); RS number, reference single nucleotide variants number; SIFT, Sorting Intolerant from Tolerant.

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Table 3 Clinical characteristics and details of patients with genetic variants (n = 45).

Family	Age at enrollment (years)	Sex	At diagnosis			Thyroid scintigraphy/USG	Transient or permanent	Genetic variant information			
			Age (years)	FT_4 (ng/dL)	TSH (mU/L)			Genes	Variants		Zygosity
1	17.8	F	NBS	1.9	21.5	Eutopic	Permanent	*DUOX2*	c.2048G>T (p.Arg683Leu), c.4027C>T (p.Leu1343Phe)	c.2635G>A (p.Glu879Lys)	ComHet
2	3.1	F	NBS	0.3	>100.0	Eutopic	Permanent	*DUOX2*	c.2654G>T (p.Arg885Leu)	c.2895_2898delGTTC (p.Phe966Serfs*29)	ComHet
3	11.7	M	NBS	1.0	>50.0	NA	Permanent	*DUOX2*	c.1310G>C (p.Gly437Ala)	c.3115C>T (p.Arg1039Trp)	ComHet
4	17.0	M	NBS	0.7	>100.0	Eutopic	Permanent	*DUOX2*	c.1588A>T (p.Lys530Ter)	c.3631C>T (p.Arg1211Cys)	ComHet
5	16.8	M	NBS	T_4 4.5 µg/dL (N, 6-15)	>50.0	Eutopic	Permanent	*DUOX2*	c.1304A>G (p.Asp435Gly)	c.1588A>T (p.Lys530Ter)	ComHet
6	17.4	M	NBS	T_4 2.8 µg/dL (N, 6-15)	48.2	Eutopic	Permanent	*DUOX2*	c.1588A>T (p.Lys530Ter)	c.2101C>T (p.Arg701Ter)	ComHet
7	12.7	F	NBS	0.3	>100.0	NA	Permanent	*DUOX2*	c.2654G>T (p.Arg885Leu)	c.3329G>A (p.Arg1110Gln)	ComHet
8	18.6	F	NBS	0.5	>100.0	NA	Permanent	*DUOX2*	c.1310G>C (p.Gly437Ala)	c.3478_3480delCTG (p.Leu1160del)	ComHet
9	17.5 (1st twin)	M	NBS	0.9	31.0	NA	Permanent	*DUOX2*	c.1588A>T (p.Lys530Ter)	c.2654G>A (p.Arg885Gln)	ComHet
	17.5 (2nd twin)	M	NBS	0.5	41.0	NA	Permanent	*DUOX2*	c.1588A>T (p.Lys530Ter)	c.2654G>A (p.Arg885Gln)	ComHet
10	11.1	F	NBS	NA	NA	NA	Permanent	*DUOX2*	c.1588A>T (p.Lys530Ter)	c.1588A>T (p.Lys530Ter)	Hom
11	6.2	F	NBS	0.4	>100.0	Eutopic	Permanent	*DUOX2*	c.3693+1G>T	WT	Het
12	3.1	M	NBS	0.9	60.8	Eutopic	Permanent	*DUOX2*	c.3340delC (p.Leu1114Serfs*56)	WT	Het
13	11.7	M	NBS	1.2	10.3	NA	Permanent	*DUOX2*	c.1295G>A (p.Arg432His)	WT	Het
14	20.8	M	NBS	0.9	>50.0	Eutopic	Permanent	*DUOX2*	c.2048G>T (p.Arg683Leu), c.4027C>T (p.Leu1343Phe)	WT	Het
15	17.2	M	NBS	1.5	17.7	NA	Permanent	*DUOX2*	c.4408C>T (p.Arg1470Trp)	WT	Het
16	5.0	M	NBS	0.4	>100.0	NA	Transient	*DUOX2*	c.2048G>T (p.Arg683Leu), c.4027C>T (p.Leu 1343Phe)	c.2654G>A (p.Arg885Gln)	ComHet
17	13.4	M	NBS	T_4 1.5 µg/dL (N, 6-15)	>50.0	Eutopic	Transient	*DUOX2*	c.2654G>A (p.Arg885Gln)	c.3693+1G>T	ComHet
18	6.3	F	NBS	1.4	7.1	Eutopic	Transient	*DUOX2*	c.1304A>G (p.Asp435Gly)	c.4080G>T (p.Lys1360Asn)	ComHet
19	4.2	M	NBS	0.5	>100.0	Eutopic	Transient	*DUOX2*	c.1232G>A (p.Arg411Lys)	WT	Het
20	8.4	F	NBS	T_4 4.1 µg/dL (N, 6-15)	45.1	Eutopic	Transient	*DUOX2*	c.2101C>T (p.Arg701Ter)	WT	Het
21	1.1 (1st twin)	M	NBS	0.9	38.9	Eutopic	Unknown[e]	*DUOX2*	c.1588A>T (p.Lys530Ter)	c.2104_2106delGGA (p.Gly702del)	ComHet
	1.1 (2nd twin)	M	NBS	0.8	92.7	Eutopic	Unknown[e]	*DUOX2*	c.1588A>T (p.Lys530Ter)	c.2104_2106delGGA (p.Gly702del)	ComHet
22	0.1	F	NBS	0.5	>100.0	NA	Unknown[e]	*DUOX2*	c.1588A>T (p.Lys530Ter)	c.2654G>T (p.Arg885Leu)	ComHet
23	0.4	F	0.2[a]	0.6	>100.0	NA	Unknown[e]	*DUOX2*	c.989T>G (p.Val330Gly)	c.2104_2106delGGA (p.Gly702del)	ComHet

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24	8.4	F	NBS	T_4 2.7 µg/dL (N, 6-15)	>50.0	NA	Permanent	*DUOXA2*	c.738C>G (p.Tyr246Ter)	c.738C>G (p.Tyr246Ter)	Hom
25	7.3	M	NBS	0.4	>100.0	NA	Permanent	*DUOXA2*	c.232G>A (p.Val78Met)	WT	Het
26	28.1	F	5[b]	NA	NA	Ectopic	Permanent	*DUOXA2*	c.738C>G (p.Tyr246Ter)	WT	Het
27	5.5	F	NBS	0.5	>100.0	Eutopic	Transient	*DUOXA2*	c.604G>A (p.Ala202Thr)	c.738C>G (p.Tyr246Ter)	ComHet
	2.2	M	0.1[a]	0.6	>100.0	NA	Unknown[e]	*DUOXA2*	c.604G>A (p.Ala202Thr)	c.738C>G (p.Tyr246Ter)	ComHet
28	5.6	M	0.1[a]	0.1	>100.0	Eutopic	Transient	*DUOXA2*	c.501C>A (p.Cys167Ter)	WT	Het
29	6.2	M	NBS	0.7	>100.0	NA	Permanent	*TG*	c.274+2T>G	c.1348delT (p.Ser450Profs*29)	ComHet
30	14.6	M	NBS	0.7	>100.0	NA	Permanent	*TG*	c.48G>A (p.Trp16Ter)	c.6791G>A (p.Cys2264Tyr)	ComHet
31	25.5	M	8.6[c]	NA	NA	Eutopic	Permanent	*TPO*	c.670_672delGAC (p.Asp224del)	c.2422delT (p.Cys808Alafs*24)	ComHet
	24.0	F	6.7[c]	NA	NA	Eutopic	Permanent	*TPO*	c.670_672delGAC (p.Asp224del)	c.2422delT (p.Cys808Alafs*24)	ComHet
32	11.2	M	NBS	0.2	>100.0	NA	Permanent	*SLC5A5*	c.794A>G (p.Gln265Arg)	c.794A>G (p.Gln265Arg)	Hom
33	31.2	M	NBS	T_4 2 µg/dL (N, 6-15)	>100.0	NA	Permanent	*TSHR*	c.545+5G>T	c.1825C>T (p.Arg609Ter)	ComHet
	24.4	M	NBS	NA	NA	NA	Permanent	*TSHR*	c.545+5G>T	c.1825C>T (p.Arg609Ter)	ComHet
34	6.4 (1st twin)	F	NBS	0.4	>100.0	Athyreosis	Permanent	*TSHR*	c.1960A>T (p.Ile654Phe)	c.1960A>T (p.Ile654Phe)	Hom
	6.4 (2nd twin)	F	NBS	0.6	>100.0	Athyreosis	Permanent	*TSHR*	c.1960A>T (p.Ile654Phe)	c.1960A>T (p.Ile654Phe)	Hom
35	9.4	M	NBS	1.1	6.6	NA	Permanent	*TSHR*	c.1349G>A (p.Arg450His)	WT	Het
36	10.8	F	NBS	1.7	97.5	Athyreosis	Permanent	*PAX8*	c.203C>T (p.Thr68Ile)	WT	Het
37	22.5	M	19.1[d]	0.6	>100.0	NA	Permanent	*PAX8*	c.92G>A (p.Arg31His)	WT	Het
38	22.8	F	5.4[d]	0.3	>100.0	Hypoplasia	Permanent	*PAX8*	c.236C>T (p.Ser79Phe)	WT	Het
39	9.5	M	NBS	T_4 8.1 µg/dL (N, 6-15)	7.7	Absent uptake, but present thyroid on USG	Permanent	*PAX8*	c.457_458delCT (p.Leu153Glufs*47)	WT	Het

Normal range for FT_4 (ng/dL): neonates age 0–2 weeks 0.9–5.0; infants 0.8–2.1; children and adults 0.7–1.4. Normal range for TSH (mU/L): neonates age 4–7 days 1.3–16.0; infants 0.9–7.1; children and adults 0.6–4.5. To convert FT_4 in ng/dL to pmol/L, multiply by 12.9; T_4 in µg/dL to nmol/L, multiply by 12.9 and TSH in mU/L to µIU/mL multiply by 1.0.

[a]Presented with prolonged jaundice; [b]Presented with ectopic thyroid; [c]Presented with short stature and goiter; [d]Presented with short stature; [e]Less than 3 years of age, permanence awaited to be determined.

ComHet, compound heterozygous; F, female; FT_4, free thyroxine; Het, heterozygous; Hom, homozygous; M, male; N, normal range; NA, not available; NBS, newborn screening; T_4, thyroxine; TSH, thyroid-stimulating hormone; USG, ultrasonography; WT, wild type.

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DUOXA2 genes from 12 families which were inherited as an autosomal recessive manner, 22 heterozygous parents had normal FT_4, TSH and Tg concentrations, while 2 parents (Families 6 and 16) had mildly elevated Tg concentrations, but normal FT_4 and TSH (Fig. 1 and Table 4).

Some heterozygous variants of the *DUOX2* and *DUOXA2* genes have been described as an autosomal dominant inheritance. Four out of five parents who were tested and carried the same heterozygous variants as their offsprings had normal FT_4, TSH and Tg concentrations. Only the mother of a patient with *DUOX2* defect who carried two variants in the same allele (c.2048G>T and c.4027C>T) had subclinical hypothyroidism which was subsequently found to be related to autoimmune thyroiditis (Fig. 1, family 14 and Table 4).

Discussion

This study demonstrated that the frequency of genetic defects in the genes causing TDH was more common than that of the genes causing TD (36/118 (30%) vs 9/118 (8%)) which was in agreement with the previous studies (4, 5, 22, 23, 24). The most frequently affected gene in this study was *DUOX2* (25 out of 45, 56%). This finding is consistent with the frequency reported in other Asian countries (Korea, Japan and China) at 53–74% (4, 18, 19). In contrast, *TG* and *TPO* variants were demonstrated in 4 out of 45 patients (9%) which was much less than that of *DUOX2* variants. *TG* and *TPO* variants have been reported as the most frequent cause of TDH in Western populations (5, 6). The high rate of *DUOX2* variants in Asians could be explained by the founder effect which contributed to more frequent occurrence of the particular variants compared with other populations. MAF of normal control Thai database of 11 out of 22 *DUOX2* variants identified in this study was greater than that of the general population from the gnomAD (0.001–0.01 vs 0.00002–0.0007) (Table 2).

DUOX2 requires *DUOX1* and their maturation factors (*DUOXA1* and *DUOXA2*) to maintain normal hydrogen peroxide (H_2O_2) production (1, 25). Twenty-two different *DUOX2* variants (Table 2) were identified in this cohort. The c.1588A>T in *DUOX2* gene was highly recurrent in 9 out of 25 patients (36%) with *DUOX2* variants in our cohort. The c.1588A>T variant had population-specificity and was mainly reported from Asian countries (26, 27, 28). Interestingly, among these 9 patients who

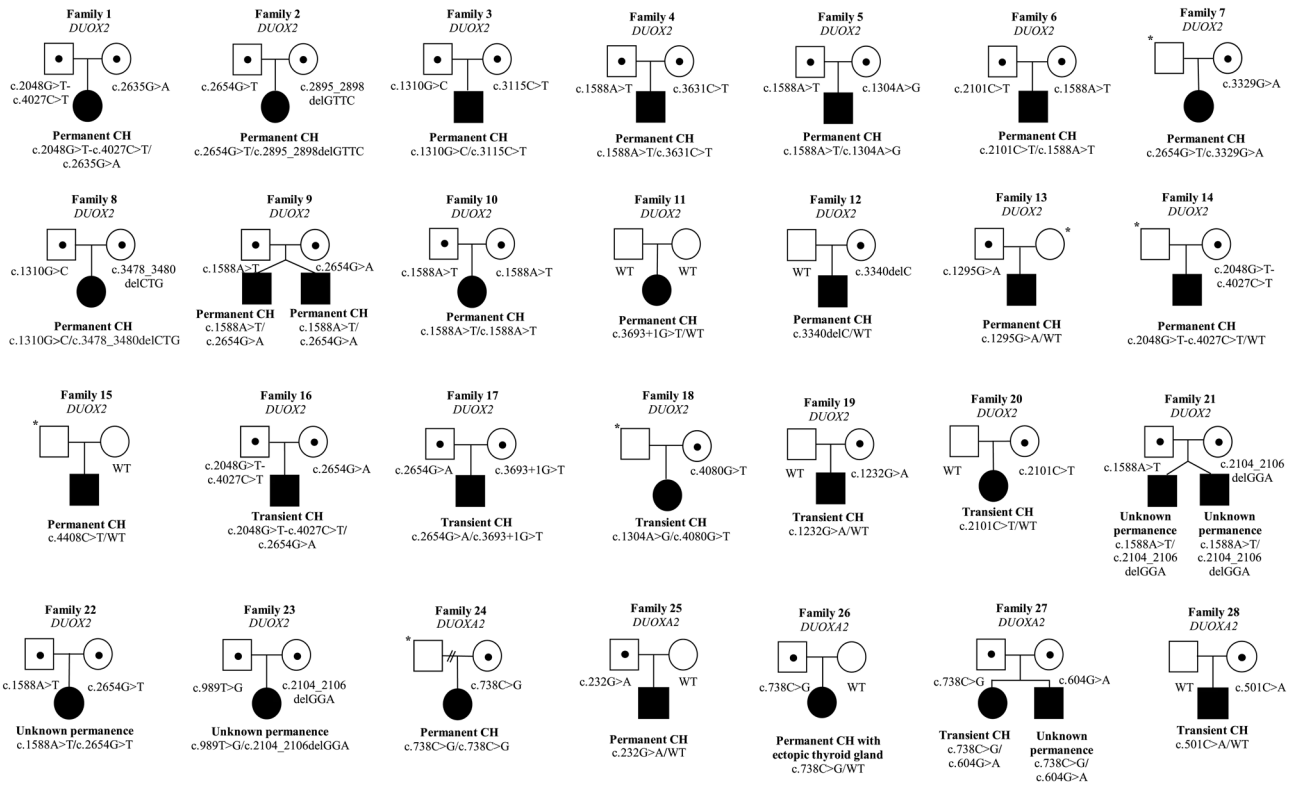

Figure 1
Pedigree of patients with *DUOX2* and *DUOXA2* variants CH, congenital primary hypothyroidism; WT, wild type; *, no DNA available.

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Table 4 Thyroid function tests of the parents of the patients with *DUOX2* and *DUOXA2* variants.

Family	Member	FT_4 (ng/dL)	TSH (mU/L)	Tg (ng/mL)
1	Father	1.0	0.8	5.6
	Mother	1.0	2.0	18.3
2	Father	ND	ND	ND
	Mother	ND	ND	ND
3	Father	0.8	1.8	9.2
	Mother	0.8	3.7	3.7
4	Father	0.9	1.6	16.7
	Mother	1.0	1.9	14.6
5	Father	0.9	1.3	14.3
	Mother	1.1	1.0	4.0
6	Father	1.1	1.7	81.2
	Mother	0.9	0.9	9.3
7	Father	ND	ND	ND
	Mother	ND	ND	ND
8	Father	0.9	0.5	5.8
	Mother	0.9	1.0	5.1
9	Father	ND	ND	ND
	Mother	ND	ND	ND
10	Father	ND	ND	ND
	Mother	ND	ND	ND
11	Father	ND	ND	ND
	Mother	ND	ND	ND
12	Father	ND	ND	ND
	Mother	ND	ND	ND
13	Father	1.0	0.5	11.7
	Mother	ND	ND	ND
14	Father	ND	ND	ND
	Mother	0.8	10.5	3.7
15	Father	ND	ND	ND
	Mother	ND	ND	ND
16	Father	1.2	1.3	8.5
	Mother	1.0	1.1	83.1
17	Father	0.9	2.7	4.6
	Mother	0.9	0.6	3.6
18	Father	ND	ND	ND
	Mother	ND	ND	ND
19	Father	ND	ND	ND
	Mother	1.0	0.6	7.3
20	Father	ND	ND	ND
	Mother	ND	ND	ND
21	Father	1.1	1.5	13.9
	Mother	0.9	2.0	7.2
22	Father	1.0	1.4	4.2
	Mother	0.8	2.5	12.0
23	Father	1.0	0.9	12.1
	Mother	1.0	1.3	38.0
24	Father	ND	ND	ND
	Mother	ND	ND	ND
25	Father	ND	ND	ND
	Mother	ND	ND	ND
26	Father	1.3	0.7	34.7
	Mother	ND	ND	ND
27	Father	0.8	0.7	7.3
	Mother	0.9	2.8	26.8
28	Father	ND	ND	ND
	Mother	T_4 7 µg/dL (N, 4-13)	1.7	ND

FT_4, free thyroxine; T_4, thyroxine; TSH, thyroid-stimulating hormone; Tg, thyroglobulin; ND, not done.
Adult normal ranges for FT_4 0–7-1.4 ng/dL, TSH 0.6–4.5 mU/L, Tg 3.5–77.0 ng/mL. To convert FT_4 in ng/dL to pmol/L, multiply by 12.9; TSH in mU/L to µIU/mL multiply by 1.0 and Tg in ng/mL to µg/L multiply by 1.0.

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carried c.1588A>T in both compound heterozygous and homozygous patterns, 6 of them had permanent CH and the remaining 3 were less than 3 years of age whose permanence awaited to be determined. Therefore, most patients with c.1588A>T variant in this study had permanent CH. However, previous studies demonstrated that the clinical phenotype of patients carrying c.1588A>T in each different genotype (biallelic and monoallelic variants) had both transient and permanent CH (27, 29). The difference in the phenotype of patients who had the same variants among studies could be explained by the difference in thyroid hormone requirement with various ages, iodine status, variable variants in the other allele and variable H_2O_2 supply by *DUOX1*/*DUOXA1* system (27). This study found double variants in the same allele (c.2048G>T and c.4027C>T) in 3 patients (Table 3, families 1, 14 and 16). Although, there was a study which demonstrated increased severity in patients who had greater number of variants (29), this study demonstrated that 2 patients with compound heterozygous variants 3 variants) had both transient and permanent CH, but the patient who had heterozygous variant (2 variants) experienced permanent CH. These heterozygous variants have never been reported as a cause of CH, so functional studies of these variants are required. Additionally, c.2895_2898delGTTC variant which was commonly reported in Western population (30), was found in only one patient in this study. Therefore, the variant frequency seemed to be ethnic specific.

Four different variants in *DUOXA2* gene were identified in this study. The nonsense variant c.738C>G was the most frequent *DUOXA2* variant. Its functional studies have already been performed (31, 32). In normal control Thai database, this variant had low MAF of 0.002. Interestingly, this variant in *DUOXA2* gene which is usually related to TDH, was found in a heterozygous pattern in the patient who had an ectopic thyroid gland (Table 3, family 26). A previous study reported an association of ectopic thyroid gland with *DUOX2* variants (33). We postulate that *DUOXA2* variants might also be related to thyroid gland development. However, the functional impact of the heterozygous c.738C>G variant in *DUOXA2* gene was not assessed, and the finding could not exclude *DUOX2* or other gene deletions.

Both parents of the patient with homozygous variants of *SLC5A5* had a heterozygous state of the variant confirmed by Sanger sequencing. This variant was not identified in our in-house Thai database (455 persons). The parents absolutely denied a history of consanguinity. The homozygous state in the patient could be caused by unrecognized consanguineous history of the family because the parents' hometown was in the northeastern region of Thailand.

Two patients with compound heterozygous *TG* variants were identified in this study. The c.274+2T>G variant found in 1 patient was a common variant reported in Chinese patients (34). Although *TG* variants have been reported as the most prevalent cause of TDH in Europeans, they were infrequent in our cohort.

In this study, the compound heterozygous, in-frame deletion (c.670_672delGAC) and frameshift mutations (c.2422delT) in *TPO* gene were identified in two siblings. Both variants have previously been reported (35, 36). Both patients developed goiter during childhood as a CH presentation which was in accordance with that reported in a Japanese patient who carried the same c.670_672delGAC variant and developed large goiter at 8 years of age (37). Retaining about 50% of residual peroxidase activity might explain the mild phenotype (35, 37). The development of multinodular goiter was possibly caused by delay in diagnosis and treatment (37, 38).

TSHR variants cause variable CH phenotypes. Hypothyroidism in our patients with either compound heterozygous or homozygous *TSHR* variants was more severe than those carrying heterozygous variant which was similar to previous reports (39, 40, 41).

PAX8 variants were inherited via autosomal dominant pattern with variable expressivity (42). Interestingly, our patient with novel c.457_458delCT variant had an absent thyroidal uptake on thyroid scintigraphy, but normal appearance of thyroid gland on ultrasonography which is a characteristic finding of iodide transport defect. Therefore, *PAX8* variants might affect sodium iodide symporter expression (43).

This study did not find the variants in the genes related to syndromic defects such as *NKX2-1, FOXE1, JAG1* and *TBX1* because the patients with obvious syndromic features and typical phenotypes were excluded from the CES analysis.

The strengths of this study include being the first relatively large study of genetic diagnosis of CH in Thai patients, having comprehensive clinical courses to be analyzed with genetic diagnosis and having thyroid function tests of heterozygous parents of the patients with *DUOX2* and *DUOXA2* variants. However, there were some limitations. First, *DUOX1* and *DUOXA1* genes which are required for full-function of *DUOX2* and *DUOXA2* genes were not included in TruSight One Sequencing Panel®. Second, patients with heterozygous variants of *DUOX2* and *DUOXA2* genes might carry undetected variants in the other allele, because NGS cannot detect a large gene deletion or variants in non-coding

regions. Third, some recently identified genetic defects causing CH which were not included in the panel used in this study such as *SLC26A7* could have been missed. In conclusion, *DUOX2* variants were the most common cause of CH among Thai patients, while *TG* and *TPO* variants were less common. The c.1588A>T in *DUOX2* gene was a common variant in this population.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement

K S, T T, P M and P P designed the work, collected, analyzed and interpreted data for the work, and drafted the article. W C, N I, I S, B P, P J and S N undertook the laboratory work, analyzed and interpreted data for the work. P K, S P, C S, M K and C S collected the data. All authors read and approved the final article.

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