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

Kinnaree Sorapipatcharoen\(^1\), Thipwimol Tim-Aroon\(^1\), Pat Mahachoklertwattana\(^1\), Wasun Chantratita\(^2\), Nareenart Iemwimangsa\(^2\), Insee Sensorn\(^2\), Bhakkhoon Panthan\(^3\), Poramate Jiaramai\(^2\), Saisuda Noojarer\(^1\), Patcharin Khlairit\(^1\), Sarunyu Pongratanakul\(^1\), Chittiwat Suprasongsin\(^5\), Manassawee Korwutthikulrangsri\(^1\), Chutintorn Sriprapradang\(^4\) and Preamrudee Poomthavorn\(^1\)

\(^1\)Department of Pediatrics, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok, Thailand
\(^2\)Center for Medical Genomics, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok, Thailand
\(^3\)Research Center, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok, Thailand
\(^4\)Department of Medicine, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok, Thailand

Correspondence should be addressed to P Poomthavorn: preamrudee.poo@mahidol.ac.th

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.

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
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, DUOX1A1, DUOX2, 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 (FT4) 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 (LT4) 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 LT4 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 I. (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 SERPINA47) was developed in-house. It covered 62 potential candidate genes (Table 1) which are

Table 1

- XX

1

- XX

2

- XX

13

- XX

12

- XX

11

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10

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- XX

5

- XX

4

- XX

3

- XX

2

- XX

1

- XX

0
| 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      | Thyroid dyshormonogenesis                                                   | 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         |
|                                    | OTA2     | 600373      | Combined pituitary hormone deficiencies                                    | AD          |
|                                    | POU1F1   | 173110      | Combined pituitary hormone deficiencies                                    | AD, AR      |
|                                    | PROP1    | 601538      | Combined pituitary hormone deficiencies                                    | AD          |
|                                    | IRS4     | 300904      | Combined pituitary hormone deficiencies                                    | AD          |
| Thyroid hormone resistance and     | THRB     | 190160      | Thyroid hormone resistance                                                  | AD, AR      |
| abnormal thyroid hormone           | THRA     | 190120      | Congenital nongoitrous hypothyroidism                                      | AD          |
| metabolism                          | SLC16A2  | 300095      | Allan-Herndon-Dudley syndrome                                               | XLR         |
| Syndromes or transcription factors  | SECISBP2 | 607693      | Abnormal thyroid hormone metabolism                                         | AR          |
| which may be associated with        | SALL1    | 602218      | Townes-Brocks syndrome                                                      | AD          |
| congenital hypothyroidism          | UBR1     | 605981      | Johanson-Blizzard syndrome                                                  | AR          |
|                                    | DYSK1A   | 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                                              | AD          |
|                                    | 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          |
|                                    | PTHRH2   | 608625      | Infantile-onset multisystem neurologic, endocrine, and pancreatic disease   | AR          |
|                                    | RYR2     | 180902      | Hyperemesis gravidarum                                                      | ND          |
|                                    | SERPINA7 | 314200      | Thyroxine binding globulin deficiency                                       | XLR         |
|                                    | SLC30A10 | 611146      | Hypermanganeseemia with dystonia                                            | AR          |
|                                    | SLC01C1  | 613389      | Thyroid hormone transporter deficiency                                      | AR          |

(Continued)
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 FT4, TSH and thyroglobulin (Tg) concentrations were determined in the heterozygous parents who carried the same DUOX2 or DUOX2A 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
genes related to TDH, including DUOX2 \((n = 25)\), DUOX2 \((n = 6)\), 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 DUOX2 variants, 2 TG variants, 1 SLC5A5 variant, 3 PAX8 variants and 2 TSHR variants \((\text{Table 2})\). 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 DUOX2 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 \((23\%)\). Eighteen out of 25 patients \((72\%)\) carried either compound heterozygous or homozygous variant; 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 DUOX2 variants were identified in 6 patients \((5\%)\), of which three of them had either compound heterozygous or homozygous variant; and the other three had heterozygous variants. The most common DUOX2 variant was c.738C>G, in 5 alleles in 4 patients. Five patients with DUOX2 variants and 2 patients with DUOX2 variants had transient CH and 16 patients with DUOX2 variants and 3 patients with DUOX2 variants had permanent CH. The remaining 4 patients with DUOX2 variants and 1 patient with DUOX2 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 DUOX2 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 DUOX2 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 LT4 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 FT4 concentrations at diagnosis of patients with monoallelic and biallelic variants were not statistically different \([\text{TSH}: 50.0 (17.7, 100.0) \text{ and } 50.0 (39.5, 100.0) \mu\text{U/L}}, p=0.604; \text{FT4: 0.9 (0.4, 1.3) and 0.6 (0.4, 0.9) ng/dL}}, p=0.482, \text{respectively})\]. There was no evidence of genotype-phenotype correlation.

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

Serum FT4, TSH and Tg concentrations were determined in 29 homozygous parents from 17 families of patients who carried variants of the DUOX2 and DUOX2 genes \((\text{Fig. 1 and Table 4})\). Regarding patients with compound heterozygous and homozygous variants in the DUOX2 and
Table 2

| Genes         | Nucleotide position | Amino acid position | Mutation types | SIFT         | Polyphen-2 | Allele frequency (gnomAD, n=455) | Thai allele frequency | Status (accession number) |
|---------------|---------------------|---------------------|----------------|--------------|------------|----------------------------------|-----------------------|--------------------------|
| DUOX2         | c.1588A>T           | p.Lys530Ter          | Nonsense       | NA           | Deleterious | 0.000675966                      | 0.00989               | Reported                 |
|               |                     |                     |                |              | NA         | rs180671269                      |                       |                          |
|               | c.2654G>A           | p.Arg885Gln          | Missense       | NA           | Deleterious | 0.006                          | 0.999                 | Reported                 |
|               |                     |                     |                |              | NA         | rs181461079                      |                       |                          |
|               | c.2048G>T           | p.Arg683Leu          | Missense       | NA           | Deleterious | 0.002                          | 1.000                 | Reported                 |
|               |                     |                     |                |              | NA         | rs80246825                       |                       |                          |
|               | c.2104_2106delGGA   | p.Gly702del          | In-frame deletion | NA           | NA         | 0.000075580                      | 0.010989              | Reported                 |
|               |                     |                     |                |              | NA         | rs779340990                      |                       |                          |
|               | c.4027C>T           | p.Leu1343Phe         | Missense       | NA           | Tolerated  | 0.054                          | 0.831                 | Reported                 |
|               |                     |                     |                |              | NA         | rs147945181                      |                       |                          |
|               | c.1304A>G           | p.Asp435Gly          | Missense       | NA           | Deleterious | 0.002                          | 1.000                 | Reported                 |
|               | c.4027C>T           | p.Leu1343Phe         | Missense       | NA           | Tolerated  | 0.054                          | 0.831                 | Reported                 |
|               | c.1310G>C           | p.Arg411Lys          | Missense       | NA           | Deleterious | 0.033                          | 0.372                 | Reported                 |
|               | c.2895_2898delGTTC  | p.Phe966Serfs*29    | Frameshift     | NA           | NA         | 0.000075555                      | 0.010989              | Reported                 |
|               | c.1295G>A           | p.Arg432His          | Missense       | NA           | NA         | rs200717240                      |                       |                          |
|               | c.2889_2898delGTTC  | p.Phe966Serfs*29    | Frameshift     | NA           | NA         | rs200717240                      |                       |                          |
|               | c.3490C>T           | p.Glu979lys          | Missense       | NA           | NA         | rs76979632                       | 0.0010989             | Reported                 |
|               | c.3396A>T           | p.Arg1150Gln         | Missense       | NA           | NA         | rs779340990                      |                       | Reported                 |
|               | c.2985_2988delGTC   | p.Phe966Serfs*29    | Frameshift     | NA           | NA         | rs779340990                      |                       | Reported                 |
|               | c.1295G>A           | p.Arg432His          | Missense       | NA           | NA         | rs200717240                      |                       |                          |
|               | c.3396A>T           | p.Arg1150Gln         | Missense       | NA           | NA         | rs779340990                      |                       | Reported                 |
|               | c.2889_2898delGTTC  | p.Phe966Serfs*29    | Frameshift     | NA           | NA         | rs779340990                      |                       | Reported                 |

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| Gene     | Variant | Phenotype | Location | Predicted Effect | p-value | rs-number | Reported/Novel | Location | Reference |
|----------|---------|-----------|----------|-----------------|---------|-----------|---------------|----------|-----------|
| DUOX2    | c.738C>G| Nonsense  | NA       | NA              | 0.001   | rs4774518 | Reported      | NA       | RSS774518 |
|          | c.604G>A| Missense  | 0.016    | Deleterious     | 0.643   | rs77014807| Novel         | SCV001 250673 | RSS77014807 |
|          | c.232G>A| Missense  | 0.076    | Tolerated       | 1.000   | rs74613285| Reported      | NA       | RSS781126484 |
|          | c.501C>A| Nonsense  | NA       | NA              | 0.00004014 | NA         | Novel         | SCV001 250908 | RSS781126484 |
| TG       | c.48G>A | Nonsense  | NA       | NA              | 0.00004367 | NA         | Reported      | NA       | RSS780846892 |
|          | c.274+2T>G| Splice site| NA       | NA              | 0.000003991 | 0.0010989 | Reported      | NA       | RSS1398373161 |
|          | c.1348delT| Frameshift| NA       | NA              | 0.00005576 | NA         | Reported      | NA       | RSS77653164 |
|          | c.6791G>A| Missense  | 0.001    | Deleterious     | 1.000   | rs722164623 | Reported      | NA       | RSS763662774 |
| TPO      | c.670_672delGAC| In-frame deletion| NA       | NA              | 0.000059679 | NA         | Reported      | NA       | RSS104893657 |
| SLC5A5   | c.2422delIT| Frameshift| NA       | NA              | 0.000083532 | NA         | Reported      | NA       | RSS104893657 |
| PAX8     | c.92G>A | Missense  | 0.000    | Deleterious     | 1.000   | rs767239688 | Novel         | SCV001 250738 | RSS767239688 |
|          | c.203C>T| Missense  | 0.000    | Deleterious     | 1.000   | NA         | Reported      | NA       | RSS763679435 |
|          | c.236C>T| Missense  | 0.000    | Deleterious     | 1.000   | NA         | Reported      | NA       | RSS189261858 |
|          | c.457_458delCT| Frameshift| NA       | NA              | 0.00001929 | 0.0021978 | Novel         | SCV001 250733 | RSS767239688 |
| TSHR     | c.1960A>T| Missense  | 0.000    | Deleterious     | 1.000   | NA         | Reported      | NA       | RSS763679435 |
|          | c.545+5G>T| Splice site| NA       | NA              | 0.00003978 | NA         | Reported      | NA       | RSS189261858 |
|          | c.1825C>T| Nonsense  | NA       | NA              | 0.0000234637 | 0.0021978 | Reported      | NA       | RSS189261858 |
|          | c.1349G>A| Missense  | 0.000    | Deleterious     | 1.000   | NA         | Reported      | NA       | RSS189261858 |

- Absent in database; 
- c.989T>G variant was predicted to be probably deleterious (0.960) by Mutation Taster; 
- 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.
## Table 3 Clinical characteristics and details of patients with genetic variants (n = 45).

| Family | Age at enrollment (years) | Sex | Age at diagnosis (years) | FT<sub>4</sub> (ng/dL) | TSH (mU/L) | Thyroid scintigraphy/USG | Transient or permanent | Genes | Variants | Genetic variant information |
|--------|--------------------------|-----|--------------------------|------------------------|------------|--------------------------|------------------------|-------|----------|-----------------------------|
| 1      | 17.8                     | F   | NBS                      | 1.9                    | 21.5       | Eutopic                  | Permanent             | DUOX2 | c.2048G>T (p.Arg683Leu), c.2654G>A (p.Glu879Lys) | ComHet |
| 2      | 3.1                      | F   | NBS                      | 0.3                    | >100.0     | Eutopic                  | Permanent             | DUOX2 | c.2654G>T (p.Arg885Leu) | ComHet |
| 3      | 11.7                     | M   | NBS                      | 1.0                    | >50.0      | NA                       | Permanent             | DUOX2 | c.1310G>C (p.Gly437Ala) | ComHet |
| 4      | 17.0                     | M   | NBS                      | 0.7                    | >100.0     | Eutopic                  | Permanent             | DUOX2 | c.1358A>T (p.Lys530Ter) | ComHet |
| 5      | 16.8                     | M   | NBS                      | 4.5 µg/dL (N, 6-15)   | >50.0      | Eutopic                  | Permanent             | DUOX2 | c.1304A>G (p.Asp435Gly) | ComHet |
| 6      | 17.4                     | M   | NBS                      | 2.8 µg/dL (N, 6-15)   | 48.2       | Eutopic                  | Permanent             | DUOX2 | c.1588A>T (p.Lys530Ter) | ComHet |
| 7      | 12.7                     | F   | NBS                      | 0.3                    | >100.0     | NA                       | Permanent             | DUOX2 | c.2654G>T (p.Arg885Leu) | ComHet |
| 8      | 18.6                     | F   | NBS                      | 0.5                    | >100.0     | NA                       | Permanent             | DUOX2 | c.1310G>C (p.Gly437Ala) | ComHet |
| 9      | 17.5                      | M   | NBS                      | 0.9                    | 31.0       | NA                       | Permanent             | DUOX2 | c.1588A>T (p.Lys530Ter) | ComHet |
| 10     | 11.1                      | M   | NBS                      | NA                     | NA         | NA                       | Permanent             | DUOX2 | c.1588A>T (p.Lys530Ter) | ComHet |
| 11     | 6.2                       | F   | NBS                      | 0.4                    | >100.0     | Eutopic                  | Permanent             | DUOX2 | c.3693+1G>T           | WT     |
| 12     | 3.1                       | M   | NBS                      | 0.9                    | 60.8       | Eutopic                  | Permanent             | DUOX2 | c.3340delC            | WT     |
| 13     | 11.7                      | M   | NBS                      | 1.2                    | 10.3       | NA                       | Permanent             | DUOX2 | c.1295G>A (p.Arg432His) | WT     |
| 14     | 20.8                      | F   | NBS                      | 0.9                    | >50.0      | Eutopic                  | Permanent             | DUOX2 | c.2048T>G (p.Arg683Leu) | WT     |
| 15     | 17.2                      | M   | NBS                      | 1.5                    | 17.7       | NA                       | Permanent             | DUOX2 | c.2048T>G (p.Arg683Leu) | WT     |
| 16     | 5.0                       | M   | NBS                      | 0.4                    | >100.0     | NA                       | Transient             | DUOX2 | c.4027C>T (p.Asp1343Phe) | WT     |
| 17     | 13.4                      | M   | NBS                      | 1.5 µg/dL (N, 6-15)   | >50.0      | Eutopic                  | Transient             | DUOX2 | c.2654G>A (p.Arg885Gln) | WT     |
| 18     | 6.3                       | F   | NBS                      | 1.4                    | 7.1        | Eutopic                  | Transient             | DUOX2 | c.1304A>G (p.Asp435Gly) | WT     |
| 19     | 4.2                       | M   | NBS                      | 0.5                    | >100.0     | Eutopic                  | Transient             | DUOX2 | c.1232G>A (p.Asp411Lys) | WT     |
| 20     | 8.4                       | F   | NBS                      | 4.1 µg/dL (N, 6-15)   | 45.1       | Eutopic                  | Transient             | DUOX2 | c.2101C>T (p.Arg701Ter) | WT     |
| 21     | 1.1 (1<sup>st</sup> twin) | M   | NBS                      | 0.9                    | 38.9       | Eutopic                  | Unknown               | DUOX2 | c.1588A>T (p.Lys530Ter) | ComHet |
| 22     | 0.1                       | F   | NBS                      | 0.5                    | >100.0     | NA                       | Unknown               | DUOX2 | c.1588A>T (p.Lys530Ter) | ComHet |
| 23     | 0.4                       | F   | NBS                      | 0.2<sup>a</sup>        | 6.2        | NA                       | Unknown               | DUOX2 | c.987T>G (p.Val330Gly) | ComHet |
## Genetics of congenital hypothyroidism

K Sorapipatcharoen et al. Genetics of congenital hypothyroidism

| No. | Age (wk) | Gender | Newborn Screening | T<sub>4</sub> (µg/dL) | T<sub>4</sub> (μg/dL) | T<sub>4</sub> (mU/L) | Gene/Variant | Clinical Features |
|-----|----------|--------|-------------------|----------------------|---------------------|-------------------|-------------|------------------|
| 24  | 8.4      | F      | NBS               | T<sub>4</sub> 2.7 (N, 6-15) | >50.0               | NA                | Permanent    | DUOXA2 c.738C>G (p.Tyr246Ter) | 1.129          |
| 25  | 7.3      | M      | NBS               | T<sub>4</sub> 0.4 (N, 6-15) | >100.0              | NA                | Permanent    | DUOXA2 c.232G>A (p.Val78Met) | 1.129          |
| 26  | 28.1     | F      | NBS               | T<sub>4</sub> NA (N, 6-15) | NA                  | NA                | Ectopic     | DUOXA2 c.738C>G (p.Tyr246Ter) | 1.129          |
| 27  | 5.5      | F      | NBS               | T<sub>4</sub> 0.5 (N, 6-15) | >100.0              | NA                | Transient   | DUOXA2 c.670_672delGAC (p.Asp224del) | 1.129          |
| 28  | 5.6      | M      | 0.1<sup>a</sup>    | T<sub>4</sub> 0.1 (N, 6-15) | >100.0              | NA                | Transient   | DUOXA2 c.501C>A (p.Cys167Ter) | 1.129          |
| 29  | 6.2      | M      | NBS               | T<sub>4</sub> 0.7 (N, 6-15) | >100.0              | NA                | Permanent    | TSHR c.1349G>A (p.Arg450His) | 1.129          |
| 30  | 14.6     | M      | NBS               | T<sub>4</sub> 0.7 (N, 6-15) | >100.0              | NA                | Permanent    | TG c.48G>A (p.Trp16Ter) | 1.129          |
| 31  | 25.5     | M      | 8.6<sup bambino</sup> | T<sub>4</sub> NA (N, 6-15) | NA                  | NA                | Eutopic     | TPO c.501C>A (p.Cys167Ter) | 1.129          |
| 32  | 11.2     | M      | NBS               | T<sub>4</sub> 0.2 (N, 6-15) | >100.0              | NA                | Permanent    | SLC5A5 c.794A>G (p.Gln265Arg) | 1.129          |
| 33  | 31.2     | M      | NBS               | T<sub>4</sub> 2.0 (N, 6-15) | >100.0              | NA                | Permanent    | TSHR c.545+5G>T | 1.129          |
| 34  | 6.4      | F      | NBS               | T<sub>4</sub> 0.4 (N, 6-15) | >100.0              | NA                | Permanent    | TSHR c.1960A>T (p.Asp654Tyr) | 1.129          |
| 35  | 9.4      | M      | NBS               | T<sub>4</sub> 1.1 (N, 6-15) | >100.0              | NA                | Permanent    | TSHR c.1349G>A (p.Arg450His) | 1.129          |
| 36  | 10.8     | F      | NBS               | T<sub>4</sub> 1.7 (N, 6-15) | 97.5                | NA                | Permanent    | TSHR c.230G>T (p.Pro68Leu) | 1.129          |
| 37  | 22.5     | M      | 19.1<sup>bambino</sup> | T<sub>4</sub> 0.6 (N, 6-15) | >100.0              | NA                | Permanent    | TSHR c.92G>A (p.Asp31His) | 1.129          |
| 38  | 22.8     | F      | 5.4<sup>bambino</sup> | T<sub>4</sub> 0.3 (N, 6-15) | >100.0              | NA                | Permanent    | TSHR c.236C>T (p.Ser79Pro) | 1.129          |
| 39  | 9.5      | M      | NBS               | T<sub>4</sub> 8.1 (N, 6-15) | 7.7                 | NA                | Permanent    | TSHR c.457_458delCT (p.Leu153Glufs*47) | 1.129          |

Normal range for FT<sub>4</sub> (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<sub>4</sub> in ng/dL to pmol/L, multiply by 12.9; T<sub>4</sub> in µg/dL to nmo/L, multiply by 12.9 and TSH in mU/L to µIU/mL, multiply by 1.0.

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

ComHet, compound heterozygous; F, female; FT<sub>4</sub>, free thyroxine; Het, heterozygous; Hom, homozygous; M, male; NA, not available; NBS, newborn screening; T<sub>4</sub>, thyroxine; TSH, thyroid-stimulating hormone; USG, ultrasonography; WT, wild type.
DUOX2 genes from 12 families which were inherited as an autosomal recessive manner, 22 heterozygous parents had normal FT₄, TSH and Tg concentrations, while 2 parents (Families 6 and 16) had mildly elevated Tg concentrations, but normal FT₄ and TSH (Fig. 1 and Table 4).

Some heterozygous variants of the DUOX2 and DUOX2A 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 offspring had normal FT₄, 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 (DUOX1A and DUOX2A) to maintain normal hydrogen peroxide (H₂O₂) 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

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**Figure 1**
Pedigree of patients with DUOX2 and DUOX2A variants CH, congenital primary hypothyroidism; WT, wild type; *, no DNA available.
Table 4  Thyroid function tests of the parents of the patients with DUOX2 and DUOXA2 variants.

| Family | Member | FT₄ (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 | 1.0         | 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 | T₄, 7 µg/dL (N, 4-13) | 1.7 | ND          |

FT₄, free thyroxine; T₄, thyroxine; TSH, thyroid-stimulating hormone; Tg, thyroglobulin; ND, not done.

Adult normal ranges for FT₄ 0–7.1.4 ng/dL, TSH 0.6–4.5 mU/L, Tg 3.5–77.0 ng/mL. To convert FT₄ 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.
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 H2O2 supply by DUOX1/DUOXA2 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 DUOX2 gene were identified in this study. The nonsense variant c.738C>G was the most frequent DUOX2 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 DUOX2 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 DUOX2 variants might also be related to thyroid gland development. However, the functional impact of the heterozygous c.738C>G variant in DUOX2 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.
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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