

Genotype-phenotype correlations of dysmorphonogenetic goiter in children and adolescents from South India

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ABSTRACT

Background: Dysmorphonogenetic goiter is one of the most common causes of hypothyroidism in children and adolescents in iodine nonendemic areas. The exact genotype-phenotypic correlations (GPCs) and risk categorization of hypothyroid phenotypes of dysmorphonogenetic mutations are largely speculative. The genetic studies in pediatric dysmorphonogenesis are very sparse from Indian sub-continent. In this context, we analyzed the implications of TPO, NIS, and DUOX2 gene mutations in hypothyroid children with dysmorphonogenetic hypothyroidism (DH) from South India. **Materials and Methods:** This is interdisciplinary prospective study, we employed eight sets of primers and screened for 142 known single nucleotide polymorphisms in TPO, NIS, and DUOX2 genes. The subjects were children and adolescents with hypothyroidism due to dysmorphonogenetic goiter. Congenital hypothyroidism, iodine deficiency, and Hashimoto's thyroiditis cases were excluded. **Results:** We detected nine mutations in 8/22 (36%) children. All the mutations were observed in the intronic regions of NIS gene and none in TPO or DUOX2 genes. Except for bi-allelic, synonymous polymorphism of TPO gene in child number 14, all other mutations were heterozygous in nature. GPCs show that our mutations significantly expressed the phenotypic traits such as overt hypothyroidism, goiter, and existence of family history. Other phenotypic characters such as sex predilection, the age of onset and transitory nature of hypothyroidism were not significantly affected by these mutations. **Conclusion:** NIS gene mutations alone appears to be most prevalent mutations in DH among South Indian children and these mutations significantly influenced phenotypic expressions such as severity of hypothyroidism, goiter rates, and familial clustering.

Key words: DUOX gene, dysmorphonogenetic goiter, hypothyroidism, NIS gene, TPO gene

INTRODUCTION

Dysmorphonogenetic hypothyroidism (DH) is a specific form of hypothyroidism caused by genetic defects in proteins involved in multiple steps of thyroid hormone biosynthesis.^[1] It is one of the commonest causes of

hypothyroidism in children and adolescents along with Hashimoto's thyroiditis (HT) and Iodine deficiency.^[2-4] DH in children appears to be a genetically determined disorder with milder defects in thyroid hormone biosynthesis leading to the delayed presentation in children and adolescents.^[5] Severe defects in thyroid hormone production presents itself as congenital or neonatal hypothyroidism or cretinism (a severe form of congenital hypothyroidism [CH]).

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Cite this article as: Ramesh BG, Bhargav PR, Rajesh BG, Devi NV, Vijayaraghavan R, Varma BA. Genotype-phenotype correlations of dysmorphonogenetic goiter in children and adolescents from South India. Indian J Endocr Metab 2016;20:816-24.

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DOI:
10.4103/2230-8210.192923

The genetic analysis in CH reveals two main groups. The first group of genes – thyroid stimulating hormone receptor (*TSHR*), thyroid transcription factor (*TTF*)-1, *TTF*-2, *Pax*-8 leads to thyroid dysgenesis with defective iodide organification. The second group of genes – *TPO*, thyroglobulin (*TG*), *NIS*, *DUOX*-2 leads to dysmorphogenesis.^[6] We are dealing only with DH cases in this study. Most of these genetic mutations appear to be inherited recessively presenting as Inborn errors of metabolism.^[6] However, literature on the etiopathogenesis and natural history of DH is largely an extrapolation of CH and only few smaller studies specifically addressed DH in older children and adolescents. Natural history of hypothyroidism in older children appears to be different from CH. DH is often self-limiting, or its hypothyroidism has waxing and waning course compared to CH, which is often associated with neurological defects and other syndromic associations.^[7,8] Further, the genetic studies in pediatric DH (noncongenital) are very sparse from Indian subcontinent. In this context, we devised this study to address the genetic basis and the clinical implication of dysmorphogenetic mutations in child and adolescent DH from South India.

MATERIALS AND METHODS

This study was conducted by collaboration between a Tertiary Care Endocrinology Hospital, Biochemistry Department of a Teaching Medical Institute and Genetics Lab. The study was conducted over the period of 36 months from August 2012 to July 2015. Ethical clearance was obtained for this study from Institutional Ethical Committee of the institute. Written informed consent was obtained from the attendants/guardians of the children included in this study. We ensured that the study complies with international ethical norms according to Helsinki declaration-Ethical Principles for Medical Research Involving Human Subjects. The diagnosis of DH was based on the exclusion of HT and iodine deficiency with demonstration of normal anti-TPO antibody titer and urinary iodine levels. In goitrous subjects, fine needle aspiration cytology (FNAC) findings of hypercellularity, colloid, and macrofollicles without lymphocytic infiltrate and Hurthle cells served as additional diagnostic criteria. Pathological, sonographic and scintigraphic findings served us in ruling out other causes of hypothyroidism. Simplified WHO criteria was employed to grade goiter size.^[9] This study included 42 hypothyroid children between 5 and 18 years. Exclusion criteria were malignant goiters, nodular goiters, and CH cases. Detailed clinical history, consanguinity and family history of thyroid disease was documented in a structured proforma. After the diagnosis

of hypothyroidism, patients were started on thyroxine replacement at the rate of 4 µg/kg/day with three monthly clinical and biochemical (with serum TSH) evaluation for titrating the dose of thyroxine to maintain euthyroidism. Thyroid scintigraphy was performed before starting on thyroxine replacement or after suspending treatment for 30 days (if already on thyroxine therapy). Twenty minutes after an intravenous injection of 10 mCi of ^{99m}Tc-pertechnetate, the images were obtained on an automated scintillation camera equipped with low-energy high-resolution parallel hole collimator. Uptake was assessed on the visual basis and no dosimetry was done. All the cases were followed for a minimum of 18 months. Serum was collected for genetic analysis of both known and unknown mutations. The detailed study protocol is given below.

Sample collection, DNA isolation, and amplification

Three milliliters of the early morning venous sample is collected from the included children in a sterile silicon coated glass tube. 50 µl, 25 µl, 200 µl, and 30 µl of serum samples were used for tri-iodothyronine (T3), thyroxine (T4), TSH, and anti-TPO assay immediately. Five milliliters of blood was collected from forearm in an ethylenediaminetetraacetic acid vial for genetic analysis and stored at -20°C. DNA was extracted from whole blood using genomic DNA isolation kit using salting out method according to manufacturer's instructions. DNA quality was checked by UV absorption at 260 and 280 nm by agarose gel electrophoresis for any degradation or RNA contamination. DNA was quantified by spectrophotometric method with absorbance at 260 nm and 280 nm [Figure 1]. The isolated DNA samples were stored at 4°C for genetic analysis later.

Polymerase chain reaction (PCR) was used to amplify *NIS* gene, *TPO* gene, and *DUOX2* gene fragments of isolated DNA. Cycling conditions were 95°C for 5 min (one cycle); at 95°C for 40 s, at 55°C for 40 s, at 72°C for 60 s (for 35 cycles), and final extension at 72°C for 10 min using one pair of primers annealing at regions of interest. We used eight sets of primers depending on number of screened single nucleotide polymorphisms (SNPs) – 2 for *DUOX2*; 2 for *TPO* and 4 for *NIS*. Each set consisted forward and reverse reading frames [Table 1]. The quality of PCR products was checked with agarose gel electrophoresis [Figure 2].

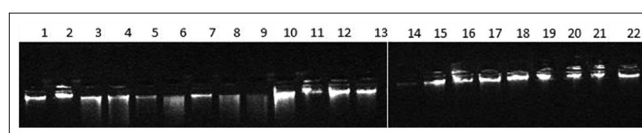


Figure 1: Qualitative check of DNA samples by agarose gel electrophoresis

Mutational analysis of *NIS*, *TPO*, *DUOX2* genes

Nucleotide sequences of all amplified PCR products were determined in both orientations by direct sequencing with an Applied Biosystems 3730XL Sequencer (Macrogen, Seoul, South Korea). The results were analyzed using Bio-Edit (version 7.1.3), (Ibis Biosciences, Carlsbad, CA, USA for Bio-Edit Applied Biosystems Co. for Sequence scanner NCBI. NLM). NIH [National Library of Medicine. National Institute of Health] Nucleotide database for Nucleotide blast program.

Two types of mutations were looked for – known (mutations already reported in the database of SNPs) and unknown (mutations never reported before). We screened for a total of 142 SNPs with the frequency distribution of 59 SNPs in *NIS* gene; 41 SNPs in *TPO* and 42 SNPs in *DUOX2* genes. Known mutations were analyzed with restriction fragment length polymorphism analysis. For unknown or

novel mutations, we planned to select hotspots on sequencing and study them. The structural and functional analysis of the gene segments consisting mutations was performed.

Laboratory reference ranges of thyroid serum biomarkers was:^[10]

- Serum total T3 = 80–180 ng/dL
- Serum total T4 = 4.5–12.6 µg/dL
- Serum TSH = 0.4–4.2 µIU/L
- Serum anti-thyroid peroxidase antibody titer = 0–34 IU/L.

The biomarkers were assayed with Siemens ADVIA Centaur CP auto-analyzer employing electro-chemiluminescent immunoassay. TSH was measured with two site sandwich immunoassay and T3, T4, anti-TPO titers were measured by competitive immunoassay. To study the dietary iodine status, urinary iodine values were measured by modified Sandell and Kolthoff method using ammonium persulfate.^[11] All children had urinary iodine within the normal range as per UNICEF and ICCIDD guidelines.^[9]

Table 1: Primer sequences with their size used in this study

Primer sequence	Size of PCR (in bp)
DUOX2F1: GCTTCCAGCATAGGCTTCAC	491
DUOX2R1: AGGATTAGAAAGGCACACCCC	
DUOX2F2: TCTCCAAATTCCTCACCAC	230
DUOX2R2: GGGCAGCAACTCAAGAAC	
TPOF1: TGCTCCTCATCACCTTTTCGG	356
TPOR1: CTCGCAAATGGACGGACAGA	
TPOF2: CTGAGCCAAGAGCTGTCCT	339
TPOR2: CGTTGCTTAGAAGGCCTCAG	
NISF1: TGCTGCCAGCTTCATGTCG	298
NISR1: TTCCGTTAAAGCACCGAGGT	
NISF2: CGAGGAGCCGCTATACATTC	212
NISR2: GCCTCAGGCTTAGGGATAG	
NISF3: AGATGGTGTGGACGGTCTCT	281
NISR3: GGGTCTCACCTGAAGGACAC	
NISF4: GCAGGGGATAAAAGAGGAGG	231
NISR4: TGAGGCAGAGATGAATGGG	

PCR: Polymerase chain reaction

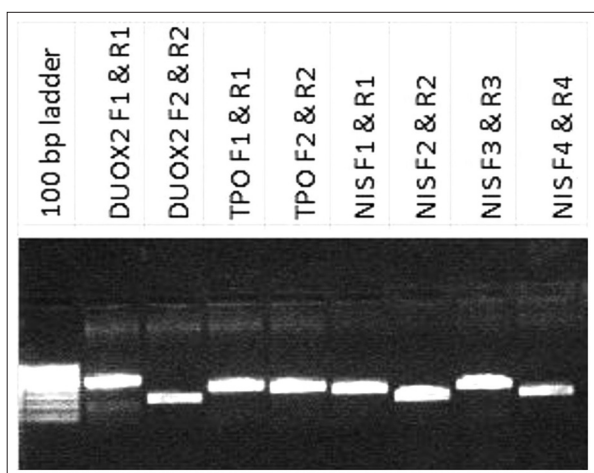


Figure 2: Qualitative analysis of polymerase chain reaction products by agarose gel electrophoresis

Statistical, specific data analysis and literature search

SPSS version 18.0 for windows (SPSS Inc., IL, USA) was employed. Descriptive analysis was done with Student's *t*-test for means and Chi-square test for categorical variables. For impact of genetic mutations of phenotypic attributes of hypothyroidism – univariate and multivariate analysis (UVA and MVA) with logistic regression for qualitative data were employed. $P < 0.05$ was considered as statistically significant.

RESULTS

Demographic, clinical, and biochemical details of all the 22 children with hypothyroidism due to DH are displayed in Table 2. female:male ratio in our cohort was 8:3 with mean age of 11.27 ± 7.1 years (5–18). Family history of hypothyroidism was present in 8/22 (36%) of patients. 11/22 (50%) were prepubertal and rested postpubertal. Mean serum TSH titers were 26.3 ± 21 mIU/mL (6.3–108). All the cases had negative or low anti-TPO antibody titers (<34 IU/L). Clinically, 17/22 (77.3%) had goitrous DH, and five children had no goiter. Grade I and II diffusely enlarged goiters were found in 5 and 12 children respectively. All the children had eutopic thyroid gland on ultrasonography and normal uptake on ^{99m}Tc scintigraphy ruling out low uptake of HT or dysgenesis. Mean follow-up of the cohort was 22.5 ± 6 (18–34) months.

Table 3 shows comparison of clinic-investigative parameters between children and adolescents. We used an arbitrary

Table 2: Clinico-investigative profile of the patients

Sex	Age (years)	Type of hypothyroidism**	Family history***	TSH (µIU/L)	Pubertal status****	Goiter grade
Female	17	A	II	34	B	1
Male	14	A	II	58	B	2
Female	10	B	I	8.5	A	2
Female	7	A	I	17	A	0
Female	10	B	II	7.5	A	2
Male	7	A	II	23	A	1
Female	12	A	II	108	A	2
Female	5	A	I	21	A	1
Female	14	A	I	16.5	B	2
Female	6	B	I	9	A	0
Male	7	A	II	45	A	2
Female	14	A	I	52	B	2
Female	17	B	II	11.5	B	0
Female	9	B	II	6.3	A	2
Male	15	A	II	19.5	B	2
Female	18	A	II	31.5	B	1
Female	8	B	I	10.5	A	0
Male	8	A	I	31	A	2
Female	13	B	II	9.5	B	1
Female	12	A	II	28.5	A	0
Male	16	A	II	16	A	2
Female	9	B	II	21	A	2

A: Overt hypothyroidism, B: Subclinical hypothyroidism, *I: Family history of hypothyroidism present, II: Absent, ****A: Prepubertal, B: Postpubertal

cut-off age limit as 12 years to differentiate children from adolescents. Children had increased frequency of subclinical hypothyroidism and positive family history amongst first degree relatives compared to adolescents. Mean serum TSH levels were significantly higher in adolescents. However, goiter rates were not significantly different.

We detected 9 *NIS* gene mutations in 8/22 (36%) children in the entire cohort. No mutations were observed in *DUOX2* and *TPO* genes. Tables 4 and 5 describe the detailed structure and topography of all the detected mutations. All these mutations were located on introns and none in exonic segments in our cohort. Except for bi-allelic, synonymous mutation of *NIS* gene in child number 14 [Figure 3], all other mutations were heterozygous in nature. Child number 12 had dual *NIS* mutations – one substitutional synonymous and another silent in nature. Child number 22 had a missense *NIS* mutation on intron. The coding annotation of this missense mutation was R (CGG) → W (TGG) in child number 22 and L (CTC) → L (CTT) for the silent mutation in child number 12. All the heterozygous mutations appear to be recessive in inheritance. Figure 4 shows a representative heterozygous *NIS* mutation corresponding to a known SNP.

Genotype-phenotypic correlations (GPCs) in Table 6 show that our mutations significantly expressed goitrous forms, overt hypothyroidism, and positive family history rates in this study. Clinical phenotypes of goiter, severity of hypothyroidism and familial clustering were statistically

Table 3: Influence of age on hypothyroidism phenotype

Variable	<12 years (n=11)	>12 years (n=11)	P
Subclinical hypothyroidism	6	2	0.03
Overt hypothyroidism	6	8	0.12
Family history	7	2	0.02
Goitrous	9	8	0.323
Mean TSH levels (mIU/L)	18.1	36.4	0.02

Student's *t*-test was used for comparing means. Chi-square with Fisher's exact correction was used for comparing frequencies. TSH: Thyroid stimulating hormone

significant in the presence of these mutations. However, they could not reach significance on multivariate analysis. Only presence of goiter in DH reached statistical significance both on UVA and MVA.

DISCUSSION

Thyroid dysmorphogenesis and dysmorphogenesis along with iodine deficiency in endemic areas and HT in late childhood are the most common causes of child hypothyroidism.^[2-4] The classical CH occurs at the rate of 1:3000–1:4000, which is mostly sporadic with familial occurrence in 2%.^[12] The two broad categories of CH are thyroid dysgenesis consisting of agenesis, hypoplasia or ectopia of thyroid gland and thyroid dysmorphogenetic goiter (DH).^[7] Genes associated with thyroid dysgenesis are *TSHR*, *TTF-1*, *Pax-8*, *TTF-2*, *NKX2-5*, which are different from those causing DH.^[6] CH is often associated with birth defects, up to 7% prevalence of nonthyroidal congenital anomalies and 1.5% of chromosomal anomalies.^[7,8] After excluding other causes of hypothyroidism in our

methodology, we included only DH cases in children above 5 years of age (noncongenital). An arbitrary cut-off age of 5 years is chosen to exclude inadvertent inclusion of late

onset CH occurring up to 4 years.^[5] About 15–20% of child hypothyroidism is due to DH.^[13] Even in India, the reported prevalence of DH was 20%.^[4] CH due to thyroid dysgenesis followed by DH is the most common cause of hypothyroidism in neonates and young children.^[13] HT is the most common cause of hypothyroidism in adults.^[2,5] Thus, late childhood and adolescence forms grey zone, wherein both DH and HT can occur. In addition, sparse data in this age group especially from India triggered us to study DH in them.

DH is a heterogenous group of thyroid disorders characterized by varying degrees of hypothyroidism due to defective thyroid hormone synthetic machinery at

Table 4: All thyroid specific mutations found in our cohort

Case number	DUOX2	TPO	NIS*
1	ND	ND	+
5	ND	ND	+
7	ND	ND	+
12	ND	ND	+
14	ND	ND	+
17	ND	ND	+
21	ND	ND	+
22	ND	ND	+

*Dual NIS mutations found in case 12. ND: No detectable mutations

Table 5: Characteristics of detected NIS mutations

Case number	Band	Location	Structural variation	Ref SNP	Allelic morphology	Summary
1	19p13.11	Chr19: 17993010	Synonymous: Intronic	rs117626343	Heterozygous	C > C/T
5	19p13.11	Chr19: 17993010	Synonymous: Intronic	rs117626343	Heterozygous	C > C/T
7	19p13.11	Chr19: 17992830	Synonymous: Intronic	rs149406361	Heterozygous	C > C/T
12	19p13.11	Chr19: 17983561	Synonymous: Intronic	rs740695	Heterozygous	G > A/G
		Chr19: 17992830	Silent: Intronic	rs149406361	Heterozygous	C > C/T
14	19p13.11	Chr19: 17983561	Synonymous: Intronic	rs740695	Homozygous	G > A/G
17	19p13.11	Chr19: 17983561	Synonymous: Intronic	rs740695	Heterozygous	G > A/G
21	19p13.11	Chr19: 17983561	Synonymous: Intronic	rs740695	Heterozygous	G > A/G
22	19p13.11	Chr19: 17992830	Mis sense: Intronic	rs149406361	Heterozygous	C > C/T

Ref SNP: Reference single nucleotide polymorphisms

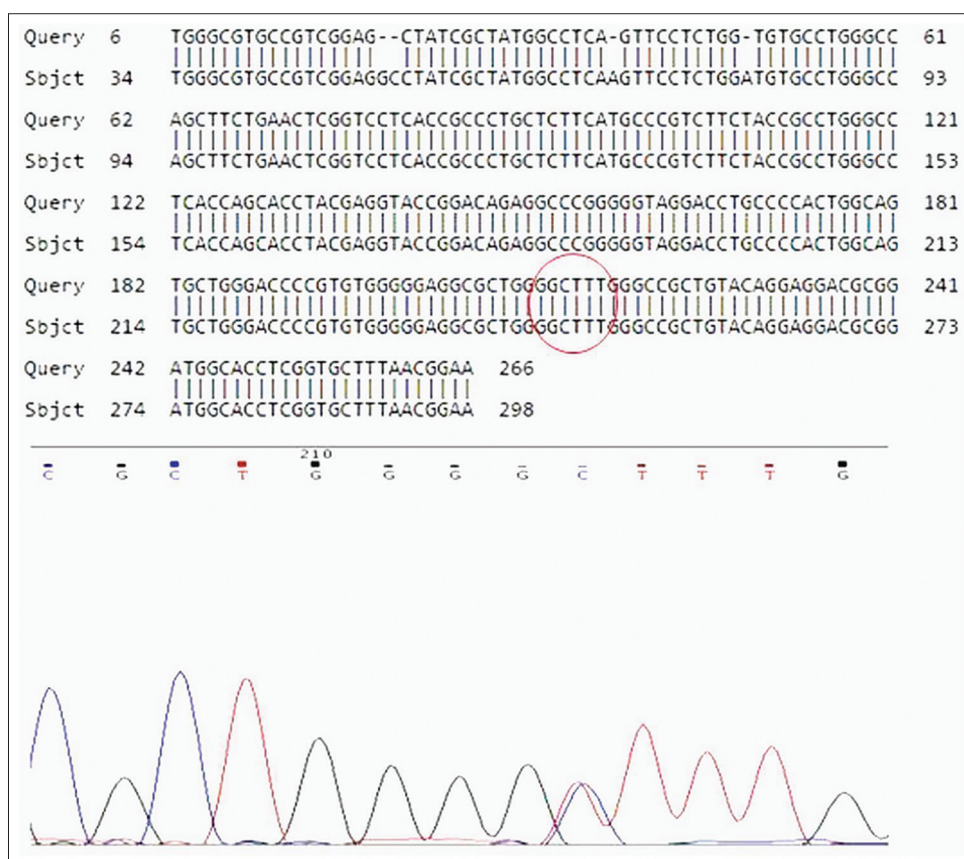


Figure 3: DNA sequencing profile and electropherogram showing NIS polymorphism with representative homozygous mutation

subcellular level.^[6] Most of the DH cases are familial with autosomal recessive mode of inheritance.^[6] Except for Pendred's syndrome (characterized by sensorineural defect along with hypothyroidism), all forms of DH are nonsyndromic and not associated with other nonthyroidal anomalies. However, we have not come across Pendred's syndrome in our cohort, as it mostly presents in early childhood. DH was synonymously and interchangeably described with wide range of terms such as – iodide transport defects (ITD), iodide organification defect (IOD), CH leading to difficulty in uniform comparison of literature

from different geographical regions.^[1,13] Ambiguity in diagnosis and exact subtyping of child hypothyroidism due to DH is caused by various bipolar clinical presentations such as syndromic or nonsyndromic; central or peripheral; primary or secondary/tertiary; permanent or transient; early or late onset; goitrous or nongoitrous; overt or subclinical forms.^[5,14] There are no robust nongenetic criteria for DH definition or diagnosis, as there is significant overlap of its clinico-investigative picture with other common causes of child hypothyroidism such as HT, iodine deficiency. Though, perchlorate discharge test (PDT) is pathognomonic of classical DH; it was not performed in our study as it was difficult to procure perchlorate and PDT has high false negative rates especially in cases with partial organification defects.^[15] Thus, diagnosis of DH in our study was based on the exclusion of other causes of hypothyroidism based on serological, biochemical, radiological, pathological, and scintigraphic findings. Thyroid uptake and scintigraphy were performed using ^{99m}Tc-perchnetate, and its proven to be more advantageous than I¹³¹ due to better quality, faster procedure, and lower radiation dose especially in

Table 6: Genotype correlation with phenotypic variables

Phenotypic variable	P	
	UVA*	MVA**
Sex (female:male)	0.131	0.734
Age (<12:>12) years	0.185	0.443
Overt versus subclinical hypothyroidism	0.03	0.22
Goitrous versus nongoitrous	0.001	0.04
Sustained versus self-limiting	0.228	0.514
Family history	0.04	0.109

*UVA: Univariate analysis, MVA: Multivariate analysis

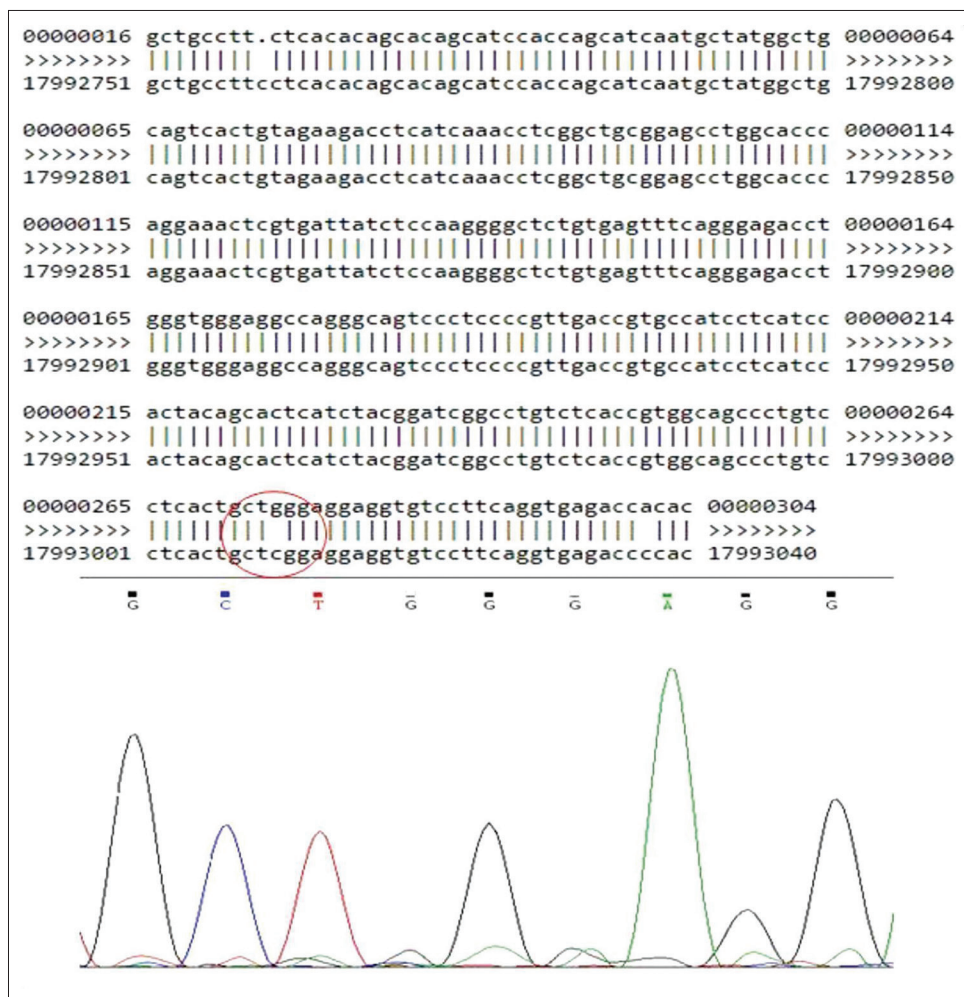


Figure 4: DNA sequencing profile and electropherogram showing NIS polymorphism with representative heterozygous mutation

children.^[16] In addition, thyroid dysgenesis and presence of normal or enlarged eutopic thyroid gland was confirmed by neck ultrasonography.

The natural history of DH in older children is usually more unpredictable compared to well-studied CH, as most of the literature on the clinical picture and natural history of child hypothyroidism is extrapolation from data on CH. In addition, DH in children and adolescents appears to be reversible in significant proportion of cases and does not correlate with goiter or onset of hypothyroidism. Pathologically, FNAC features of hypercellularity, colloid, macrofollicles with no lymphocytic infiltration were found in our goitrous DH cases. Testifying to the fact that hypothyroidism in child DH is often reversible, in our study 3 cases (13.7%) (one in overtly hypothyroid and two in subclinically hypothyroid children) were euthyroid without thyroxine therapy by the end of follow-up duration of 18 months. Probably, longer follow-up could result in more self-limiting cases of DH. Studies show that 40–50% of child HT cases with subclinical hypothyroidism are self-limiting/reversible requiring no further thyroxine replacement on long-term follow-up.^[5,17,18]

Various mutations in *NIS*, *TPO*, *DUOX2*, *TG*, *IYF/DEHAL1*, and *PDS* genes have been primarily implicated in causation of DH.^[19,20] Most of these mutations are nonsyndromic and familial, inherited in autosomal recessive pattern except for an occasional autosomal dominantly inherited *DUOX2* gene-based DH.^[6,14] This genetic insight, firmly established it as an inborn error of metabolism affecting the proteins involved in the cascade of thyroid hormone biosynthesis and its regulating pathways. Sodium iodide symporter (*NIS*) gene influencing *NIS* protein mediates active transport of iodide into thyroid gland, a vital and rate limiting step in thyroid hormone biosynthesis and functional maturation of thyroid gland. *TPO* is the key enzyme mediating the conversion of iodide to iodine, a mandatory step for coupling and organification in the production of thyroid hormones. Dual oxidase system is the enzymatic machinery regulating the hydrogen peroxide function for *TPO* catalyzed iodination and coupling. About 500 genetic mutations have been reported to cause DH in children so far. Many classifications for DH have been proposed based on these mutations, their clinical expression and GPCs. Most of them classify child hypothyroidism in to dysgenesis, dysmorphogenesis, hypothalamic-pituitary, and transitory hypothyroidism based on their candidate genes.^[5,20,21] DH was also classified based on radioiodine uptake and PDT in to ITD, IOD, and DH with normal PDT.^[1,22] Clinical expression, onset, and severity of hypothyroidism in DH is also dictated by the severity and expressivity of these mutations. Both

partial and total iodine organification defects caused by heterozygous and homozygous mutations respectively have been reported with *TPO* and *DUOX2* mutations.^[23,24] This mechanism is possible with *NIS* gene mutations. In our cohort, most of the mutations were heterozygous, but clinically hypothyroidism ranged from subclinical to overt; self-limiting to sustain with normal scintigraphic uptake, suggestive of partial dysmorphogenetic defects.

All the mutations found in our study were in intronic regulatory regions of *NIS* gene with known SNPs. We found no mutations in *TPO* and *DUOX2* genes in our cohort. Except for one case of synonymous, homozygous *NIS* mutation, the nature of these mutations was synonymous, heterozygous variants. We found no exonic or novel mutations. A mono-allelic defect with haploinsufficiency usually causes a milder defect in hormone synthesis leading to the delayed onset or subclinical or transient self-limiting hypothyroidism.^[14] A more severe defect such as bi-allelic, homozygous, exonic variants could lead to severe, early onset or permanent hypothyroidism as in CH or cretinism.^[15] However, not all bi-allelic mutations lead to permanent hypothyroidism.^[25] Very few human studies, especially from developing countries such as India, have reported on the structure and expression of thyroid-specific mutations in DH. Though, other studies showed that frequent *TPO* and *NIS* mutations, and to a lesser extent *DUOX2* predispose to,^[13,26] exclusive *NIS* mutations and undetectable *TPO*, *DUOX2* mutations in our cohort may be due to geographic and ethnic variations or missed cryptic *TPO*, *DUOX2* mutations.

One of the main objectives of this study was deriving hypothyroid phenotypes based on GPCs, which shows that our mutations had a remarkable impact on the phenotypic traits such as demographic, biochemical, and clinical variables. To analyze multiple phenotypic variables, we used reverse UVA and MVA with presence or absence of mutations as an outcome, to study the GPC. Our study showed that these *NIS* mutations expressed increased frequency of goitrous forms, overt hypothyroidism, and positive family history in hypothyroid children reaching statistical significance. Two case–control studies from India attempted to risk categorize hypothyroidism based on *TPO* genetic polymorphisms.^[27,28] Though, both the studies categorized their subjects based on the biochemical phenotype of anti-*TPO* antibody titer and level of serum thyroid hormones, but were performed on adult hypothyroidism with very short follow-up. To the best of our knowledge and literature search, we found no Indian study on GPC in hypothyroid children and adolescents for comparing our results. Other studies have reported the influence of genotype on the pattern,

timing, and natural history of hypothyroidism in DH, but mostly in CH setting.^[13,21,23,26,27] Though we had 36% family history of hypothyroidism in first-degree relatives, we are unsure about familial clustering of DH in the absence of their genetic analysis. Though all our mutations were in introns, existing literature justifies that synonymous mutations in noncoding regions can be phenotypically expressive by altering the splicing pattern, mRNA, tRNA affinities leading to posttranscriptional and posttranslational modification of downstream proteins.^[29] Though the exact mechanism is elusive, we speculate that above regulatory phenomena of gene function might be driving our heterozygous, synonymous intronic mutations in to influencing hypothyroid phenotypes. Predominance of single allele mutations in our study could be due to undetected cryptic mutations in other regulatory gene segments or the other allele.

Though many specific point mutations with GPCs have been reported, they may not be applicable to all populations worldwide due to unique genetic, geographic, ethnic and dietary factors.^[3,21] Polygenic inheritance also compounds this possibility of specific gene-based diagnosis. Finally, novel concept of noninheritable epigenetic and stochastic events in DH is a challenge in future.^[14] We need consistent results with comparable nomenclature from multiple centers of different populations and ethnicity to generalize DH patterns. Till then, an individualized protocol based on population-specific genetic results in respective geographical area is a practical solution. Still, we are far away from gene-specific intervention and decoding exact GPCs. A definitive molecular diagnosis of DH allows genetic counseling of family, differentiating transient from permanent hypothyroidism, predicting response of patients to Iodine supplementation or thyroxine supplementation and planning follow-up for asymptomatic carriers.^[30]

The apparent shortcomings in our study are smaller cohort, lack of control group, and inability to genetically screen the family members. It was not possible to procure an ideal control group for children of this hospital based sample and to get family history of thyroid disease in controls. The obvious strengths of this study are mean clinical follow-up of 22 months; focused study on hypothyroidism in older children and adolescents; risk categorization of hypothyroid phenotypes; GPC; exclusion of major confounding factors – iodine excess or deficiency, CH; a study first of its kind from India.

CONCLUSIONS

(1) *NIS* gene mutations alone appears to be most prevalent mutations in HT amongst South Indian children, ethnic and

environmental factors may be responsible for this pattern; (2) These mutations appears to be significantly influencing phenotypic expressions - such as severity of hypothyroidism, goiter rates and family history; (3) Larger studies are needed to specifically characterize dysmorphogenetic mutations and their respective hypothyroid phenotypes in children and adolescents.

Acknowledgments

We especially thank the children, their parents/guardians, genetics research team, the Medici college administration and Saveetha University for lending their impartial support by contributing this study.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Knobel M, Medeiros-Neto G. An outline of inherited disorders of the thyroid hormone generating system. *Thyroid* 2003;13:771-801.
2. Tunbridge WM, Evered DC, Hall R, Appleton D, Brewis M, Clark F, *et al.* The spectrum of thyroid disease in a community: The Wickham survey. *Clin Endocrinol (Oxf)* 1977;7:481-93.
3. Kochupillai N. Clinical endocrinology in India. *Curr Sci* 2000;79:1061-7.
4. Desai MP. Disorders of thyroid gland in India. *Indian J Pediatr* 1997;64:11-20.
5. Setian NS. Hypothyroidism in children: Diagnosis and treatment. *J Pediatr (Rio J)* 2007;83 5 Suppl: S209-16.
6. Park SM, Chatterjee VK. Genetics of congenital hypothyroidism. *J Med Genet* 2005;42:379-89.
7. Grant DB, Smith I, Fuggle PW, Tokar S, Chapple J. Congenital hypothyroidism detected by neonatal screening: Relationship between biochemical severity and early clinical features. *Arch Dis Child* 1992;67:87-90.
8. Law WY, Bradley DM, Lazarus JH, John R, Gregory JW. Congenital hypothyroidism in Wales (1982-1993): Demographic features, clinical presentation and effects on early neurodevelopment. *Clin Endocrinol (Oxf)* 1998;48:201-7.
9. WHO, UNICEF and ICCIDD. Indicators for Assessing Iodine Deficiency Disorders & Their Control through Salt Iodization. WHO/NUT/94.6. Geneva: WHO. 1994. p. 1-55.
10. Baloch Z, Carayon P, Conte-Devolx B, Demers LM, Feldt-Rasmussen U, Henry JF, *et al.* Laboratory medicine practice guidelines. Laboratory support for the diagnosis and monitoring of thyroid disease. *Thyroid* 2003;13:3-126.
11. Pino S, Fang SL, Braverman LE. Ammonium persulfate: A safe alternative oxidizing reagent for measuring urinary iodine. *Clin Chem* 1996;42:239-43.
12. Castanet M, Park SM, Smith A, Bost M, Léger J, Lyonnet S, *et al.* A novel loss-of-function mutation in TTF-2 is associated with congenital hypothyroidism, thyroid agenesis and cleft palate. *Hum Mol Genet* 2002;11:2051-9.
13. Szinnai G, editor. *Clinical genetics of congenital hypothyroidism*. In: *Pediatric Thyroidology*. Karger; Basel: Endocr Dev Karger Basel 2014. p. 60-78.

14. Moreno JC, de Vijlder JJ, Vulsma T, Ris-Stalpers C. Genetic basis of hypothyroidism: Recent advances, gaps and strategies for future research. *Trends Endocrinol Metab* 2003;14:318-26.
15. Ladsous M, Vlaeminck-Guillem V, Dumur V, Vincent C, Dubrulle F, Dhaenens CM, *et al.* Analysis of the thyroid phenotype in 42 patients with Pendred syndrome and nonsyndromic enlargement of the vestibular aqueduct. *Thyroid* 2014;24:639-48.
16. Ramos CD, Zantut Wittmann DE, Etchebehere EC, Tambascia MA, Silva CA, Camargo EE. Thyroid uptake and scintigraphy using ^{99m}Tc pertechnetate: Standardization in normal individuals. *Sao Paulo Med J* 2002;120:45-8.
17. Wasniewska M, Salerno M, Cassio A, Corrias A, Aversa T, Zirilli G, *et al.* Prospective evaluation of the natural course of idiopathic subclinical hypothyroidism in childhood and adolescence. *Eur J Endocrinol* 2009;160:417-21.
18. Monzani A, Prodam F, Rapa A, Moia S, Agarla V, Bellone S, *et al.* Endocrine disorders in childhood and adolescence. Natural history of subclinical hypothyroidism in children and adolescents and potential effects of replacement therapy: A review. *Eur J Endocrinol* 2012;168:R1-R11.
19. Moreno JC, Bikker H, Kempers MJ, van Trotsenburg AS, Baas F, de Vijlder JJ, *et al.* Inactivating mutations in the gene for thyroid oxidase 2 (THOX2) and congenital hypothyroidism. *N Engl J Med* 2002;347:95-102.
20. Szinnai G, Kosugi S, Derrien C, Lucidarme N, David V, Czernichow P, *et al.* Extending the clinical heterogeneity of iodide transport defect (ITD): A novel mutation R124H of the sodium/iodide symporter gene and review of genotype-phenotype correlations in ITD. *J Clin Endocrinol Metab* 2006;91:1199-204.
21. Spitzweg C, Morris JC. Genetics and phenomics of hypothyroidism and goiter due to NIS mutations. *Mol Cell Endocrinol* 2010;322:56-63.
22. Clerc J. Imaging the thyroid in children. *Best Pract Res Clin Endocrinol Metab* 2014;28:203-20.
23. Bakker B, Bikker H, Vulsma T, de Randamie JS, Wiedijk BM, De Vijlder JJ. Two decades of screening for congenital hypothyroidism in the Netherlands: TPO gene mutations in total iodide organification defects (an update). *J Clin Endocrinol Metab* 2000;85:3708-12.
24. Zamproni I, Grasberger H, Cortinovis F, Vigone MC, Chiumello G, Mora S, *et al.* Biallelic inactivation of the dual oxidase maturation factor 2 (DUOX2) gene as a novel cause of congenital hypothyroidism. *J Clin Endocrinol Metab* 2008;93:605-10.
25. Maruo Y, Takahashi H, Soeda I, Nishikura N, Matsui K, Ota Y, *et al.* Transient congenital hypothyroidism caused by biallelic mutations of the dual oxidase 2 gene in Japanese patients detected by a neonatal screening program. *J Clin Endocrinol Metab* 2008;93:4261-7.
26. Kosugi S, Okamoto H, Tamada A, Sanchez-Franco F. A novel peculiar mutation in the sodium/iodide symporter gene in Spanish siblings with iodide transport defect. *J Clin Endocrinol Metab* 2002;87:3830-6.
27. Guria S, Bankura B, Balmiki N, Pattanayak AK, Das TK, Sinha A, *et al.* Functional analysis of thyroid peroxidase gene mutations detected in patients with thyroid dysmorphogenesis. *Int J Endocrinol* 2014;2014:390121.
28. Balmiki N, Bankura B, Guria S, Das TK, Pattanayak AK, Sinha A, *et al.* Genetic analysis of thyroid peroxidase (TPO) gene in patients whose hypothyroidism was found in adulthood in West Bengal, India. *Endocr J* 2014;61:289-96.
29. Rearick D, Prakash A, McSweeney A, Shepard SS, Fedorova L, Fedorov A. Critical association of ncRNA with introns. *Nucleic Acids Res* 2011;39:2357-66.
30. Grasberger H, Refetoff S. Genetic causes of congenital hypothyroidism due to dysmorphogenesis. *Curr Opin Pediatr* 2011;23:421-8.