

## Brief Report

# Analysis of the T354P mutation of the sodium/iodide cotransporter gene in children with congenital hypothyroidism due to dysmorphogenesis

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### Abstract

**Background:** Congenital hypothyroidism (CH) due to the thyroid dysmorphogenesis is more prevalent in Iran in comparison to other countries. Sodium iodide symporter (*NIS*) is one of the plasma membrane glycoproteins that is located on the basolateral side of thyroid follicular cells and mediates active I<sup>-</sup> trapping into these cells. Playing a prominent role in thyroid hormone synthesis, *NIS* gene mutations can be a cause of permanent CH with the etiology of dysmorphogenesis. The aim of this study was to investigate the occurrence of T354P mutation of the *NIS* gene, in a group of children affected with permanent CH in Isfahan.

**Materials and Methods:** Thirty-five patients with the etiology of dysmorphogenesis, and 35 healthy children, collected between 2002 and 2011 in Isfahan Endocrine and Metabolism Research Center, were examined for T354P mutation of the *NIS* gene by direct polymerase chain reaction-sequencing method.

**Results:** No T354P mutation was detected in any of the studied children.

**Conclusions:** More subjects with confirmed iodide transport defects should be screened for detecting the frequency of different reported *NIS* gene mutations in our population.

**Key Words:** Congenital hypothyroidism, dysmorphogenesis, mutation, sodium iodide symporter gene, T354P

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## INTRODUCTION

Congenital hypothyroidism (CH) is the most common pediatric endocrine disease and a cause for preventable mental retardation.<sup>[1]</sup> Studies on different regional, state, and national screening programs, has confirmed

that the frequency of CH varies according to the geographic locations. Asian, Hispanic populations, and native Americans have higher rates of this disease, and American black population has shown to have lower rates of CH.<sup>[2]</sup> Approximately, all screening

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programs report that the incidence in females is twice as much as males. With the advent of the screening program of the newborn population in Isfahan (a central province in Iran), the incidence of CH was reported to be about 1 in 400 to 1 in 900.<sup>[3]</sup>

CH can be divided into two main groups: Permanent and transient forms, which in turn can be classified into primary, secondary, or tertiary etiologies.<sup>[4]</sup> Permanent CH is described as a persistent deficiency of the thyroid hormone that requires treatment during the lifetime.<sup>[5]</sup> Primary hypothyroidism includes problems in thyroid gland development (dysgenesis) or defects in thyroid hormone biosynthesis (dyshormonogenesis).<sup>[6,7]</sup> They account for 85% and 15% of CH, respectively.<sup>[5,6]</sup> CH is a multifactorial disease with different genetic, environmental, and autoimmune etiologies.<sup>[8-10]</sup> One of the most important environmental factors involved in the disease is iodine deficiency that has been overcome in Iran.<sup>[8-10]</sup> There has been a wide range of researches conducted on genetic factors, and various genetic mutations have been identified as a cause for this disease.<sup>[8,11]</sup>

Mutations, which impede thyroid hormone synthesis include defects in sodium iodide symporter (*NIS*), thyroid peroxidase, thyroglobulin, and pendrin genes that can cause permanent CH.<sup>[12-15]</sup>

*NIS* is one of the plasma membrane glycoproteins that is located in the basolateral side of thyroid follicular cells and mediates active I<sup>-</sup> trapping into the follicular cells. Iodide uptake in the follicular cells is the critical step for the synthesis of thyroid hormone with iodide accumulation in thyroid cells.<sup>[16]</sup> Different *NIS* mutations have been identified to have a prominent role in the etiology of I<sup>-</sup> transport defect (ITD). Till now, 13 mutations in the *NIS* gene have been reported, from which T354P mutation is the most common reported change in the *NIS* gene in CH patients.<sup>[17]</sup> A hydroxyl group in the  $\beta$ -carbon at position 354 is essential for *NIS* function. Such a hydroxyl group is present in Thr-354. In patients with T354P mutation, substitution of Pro instead of Thr at position causes the lack of I<sup>2</sup> transport, resulting in severe hypothyroidism.<sup>[18]</sup>

The aim of the present study was to check the occurrence of T354P mutation of the *NIS* gene, in a group of children affected with permanent CH in Isfahan.

## MATERIALS AND METHODS

### Patients and controls

Thirty-five children with permanent CH due to dyshormonogenesis were diagnosed and followed-up

during a screening program (2002–2011) in Isfahan Endocrine and Metabolism Research Center. Newborns with abnormal screening results were re-checked, and those with abnormal thyroxine (T<sub>4</sub>) and thyroid-stimulating hormone (TSH) levels on their second measurements (TSH >10 mIU/L and T<sub>4</sub> <6.5  $\mu$ g/dl) were diagnosed as CH patients, and received routine treatment and follow-up. Permanent and transient cases of CH were determined at the age of 3 years old by measuring TSH and T<sub>4</sub> concentrations, 4 weeks after the withdrawal of levothyroxine therapy. Patients with increased TSH levels (TSH >10 mIU/L) and decreased T<sub>4</sub> levels (T<sub>4</sub> < 6.5  $\mu$ g/dl) were grouped as permanent CH. Thyroid scan and/or ultrasound was used to determine the etiology of permanent CH patients. Children showing thyroid gland of normal size were considered as having dyshormonogenetic CH. The research was approved by the Ethics Committee of Isfahan University of Medical Sciences. Prior to participation, the patients' written informed consents were obtained from their parents. Thirty-five healthy children, who did not have any abnormal screening results of thyroid and with matching of age and sex with the case group, were included in the study as well. All selected children in the case and control groups were examined by a pediatrician (NM), and the demographic characteristics and screening findings regarding the level of TSH and T<sub>4</sub> were recorded using a questionnaire.

### Laboratory tests

Serum T<sub>4</sub> and TSH were measured by radioimmunoassay and immunoradiometric assay (IRMA) methods, respectively.

### Molecular genetic analysis

Genomic DNA was extracted from peripheral blood using the Diatom DNA Prep 100 kit (Isogen Laboratory, Russia), according to the manufacturer's instructions. The quality of DNA was verified by gel electrophoresis and its concentration was assessed by optical density at 260 nm using a spectrophotometer. Exon 9 of the *NIS* gene, containing the T354P mutation was amplified by polymerase chain reaction (PCR) with the following primers designed using Gene Runner software (version 3.02; Hastings software Inc): 5'-CTTTGCAGGACTGGGTTACC-3' and 5'-CCGAGGTTTGATGAGGTCTTC-3'. The amplicon size was 183 bp, and T354P mutation located at the nucleotide number 121 from 5' side of PCR amplicon. Each amplification mixture was performed in a total volume of 25  $\mu$ l, using 500 ng of genomic DNA, 0.2  $\mu$ M of each primer, 0.2  $\mu$ M of dNTP, 2.5  $\mu$ l of complete buffer (containing MgCl<sub>2</sub>), and 1.25 unit of DFS-Taq polymerase (BIORON, Germany). Cycling conditions were at 95 °C for 5 min (one cycle); at 95°C for 30 s, at

60°C for 30 s, at 72°C for 30 s (for 35 cycles); and final extension at 72°C for 10 min. PCR amplicons were visualized, after electrophoresis in an 1.5% agarose gel, stained with ethidium bromide, and examined under the ultraviolet light. Nucleotide sequences of all amplified PCR products were determined by direct sequencing with an Applied Biosystems 3730XL sequencer (Macrogen, South Korea).

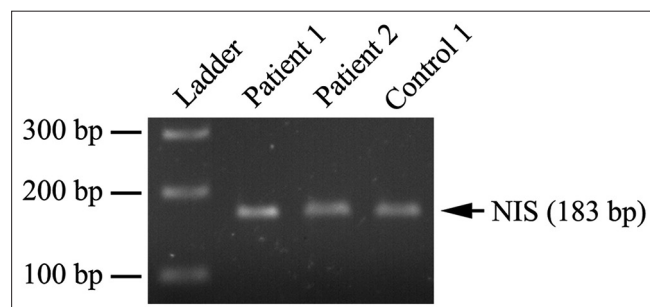
## RESULTS

In the current study, a total of 35 children with the etiology of dyshormonogenesis, and 35 healthy ones were evaluated. Demographic and laboratory findings of case and control group have been described elsewhere.<sup>[19]</sup> Specific amplification of a 183 bp amplicon of the *NIS* gene exon 9 using specific primers was detected [Figure 1]. After amplification reactions, sequencing was performed. A sample electropherogram for a part of exon 9 of the *NIS* gene in a patient and a control individual has been shown in Figure 2. Nucleotide sequences of all amplified PCR products were compared with the human *NIS* genomic sequence by BLAST online tool (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>), which showed no polymorphism in the studied population (data not shown). In overall, we did not find any T354P mutation of the *NIS* gene in the studied children.

## DISCUSSION

In the current study, the occurrence of T354P mutation of the *NIS* gene was examined in children with CH and no such mutation was found in the patients.

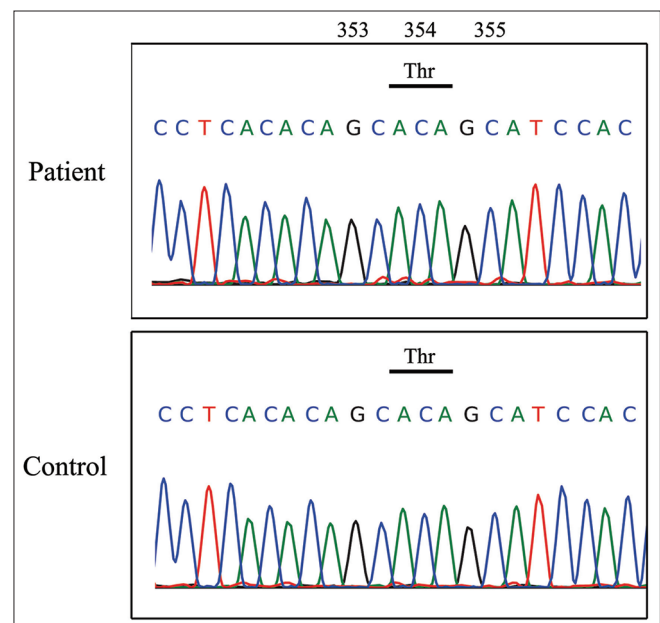
In 1997, Fujiwara *et al.* reported for the first time the T354P mutation as a cause for congenital defect of I<sup>-</sup> transport in a case report study in Japanese patients.<sup>[20]</sup> They also presented a rapid screening method to analyze the mutation without gene sequencing.<sup>[21]</sup> In 1997, Matsuda *et al.* also reported the occurrence of this mutation in a male patient



**Figure 1:** The polymerase chain reaction product using specific primers to amplify the whole size of exon 9 of the sodium iodide symporter gene. The first column represents the DNA size marker

from a consanguineous marriage. In another study by Kosugi *et al.*,<sup>[22]</sup> higher prevalence of T354P mutation in Japanese patients with ITD was reported.

ITD diagnosis is based on (a) goiter with hypothyroidism or compensated hypothyroidism, (b) little or no uptake of radioiodine, and finally (c) no concentration of iodide by salivary glands.<sup>[23]</sup> Clinical examination of our patients showed that none of them had goiter. It may be because of early diagnosis and treatment of the patients. Evidences show that the goiter may not be diagnosed in these patients at early ages of their life.<sup>[17]</sup> We did not perform radioiodine uptake assay in our patients as none of their parents allowed that. In addition, there were no facilities to test the iodide saliva-to-plasma ratio in the patients. The etiology of CH was determined mainly by thyroid scan and/or ultrasonography. Because of these reasons, it is possible that none of the examined CH patients had ITD, and therefore, they did not have a defect in the *NIS* gene. In addition, it is possible that other *NIS* mutations, rather than T354P, are present in our patients that identifying them are in our future research plans. Hence, it seems that with the accurate diagnosis of the etiology of CH in a larger sample size with a screening of the whole length of the involved genes can be helpful to determine the cause of CH in our patients. Identification of mutations in CH patients may have benefits for better managements and family genetic counseling.



**Figure 2:** Electropherograms of T354P mutation of the sodium iodide symporter gene in a sample dyshormonogenic congenital hypothyroidism patient and a control neonate

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### Conflicts of interest

There are no conflicts of interest.

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