Brief Report

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

Hajar Miranzadeh-Mahabadi[1], Modjtaba Emadi-Baygi[1,2], Parvaneh Nikpour[3,4,5], Neda Mostofizade[6], Silva Hovsepian[5,6], Mahin Hashemipour[5,6]

[1]Department of Genetics, School of Basic Sciences, Shahrekord University, [2]Institute of Biotechnology, School of Basic Sciences, Shahrekord University, Shahrekord, [3]Applied Physiology Research Center, [4]Department of Genetics and Molecular Biology, Faculty of Medicine, [5]Child Growth and Development Research Center, Research Institute for Primordial Prevention of Non-communicable Disease, [6]Department of Pediatrics, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran

Abstract

Background: Congenital hypothyroidism (CH) due to the thyroid dyshormonogenesis 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^- 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 dyshormonogenesis. 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 dyshormonogenesis, 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, dyshormonogenesis, mutation, sodium iodide symporter gene, T354P

Address for correspondence:
Dr. Parvaneh Nikpour, Department of Genetics and Molecular Biology, Faculty of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran.
E-mail: nikpour@med.mui.ac.ir

Received: 05.12.2014, **Accepted:** 13.10.2015

INTRODUCTION

Congenital hypothyroidism (CH) is the most common pediatric endocrine disease and a cause for preventable mental retardation.[1] 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.[2] Approximately, all screening

Access this article online	
Quick Response Code:	Website: www.advbiores.net
	DOI: 10.4103/2277-9175.180642

How to cite this article: Miranzadeh-Mahabadi H, Emadi-Baygi M, Nikpour P, Mostofizade N, Hovsepian S, Hashemipour M. Analysis of the T354P mutation of the sodium/iodide cotransporter gene in children with congenital hypothyroidism due to dyshormonogenesis. Adv Biomed Res 2016;5:73.

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.[3]

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

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

NIS is one of the plasma membrane glycoproteins that is located in the basolateral side of thyroid follicular cells and mediates active I^- 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.[16] Different *NIS* mutations have been identified to have a prominent role in the etiology of I^- 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.[17] A hydroxyl group in the b-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 I2 transport, resulting in severe hypothyroidism.[18]

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 (T4) and thyroid-stimulating hormone (TSH) levels on their second measurements (TSH >10 mIU/L and T4 <6.5 μg/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 T4 concentrations, 4 weeks after the withdrawal of levothyroxine therapy. Patients with increased TSH levels (TSH >10 mIU/L) and decreased T4 levels (T4 < 6.5 μg/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 T4 were recorded using a questionnaire.

Laboratory tests

Serum T4 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 μl, using 500 ng of genomic DNA, 0.2 μM of each primer, 0.2 μM of dNTP, 2.5 μl of complete buffer (containing $MgCl_2$), 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.[19] 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^- transport in a case report study in Japanese patients.[20] They also presented a rapid screening method to analyze the mutation without gene sequencing.[21] In 1997, Matsuda *et al*. also reported the occurrence of this mutation in a male patient from a consanguineous marriage. In another study by Kosugi *et al*.,[22] 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.[23] 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.[17] 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.

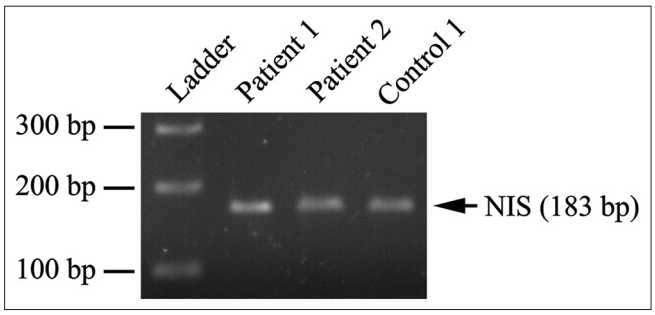

Figure 1: The polymerase chain reaction product using specific primers to amplify the whole size of exon 9 of the sodium iodide symporter gene. The first column represents the DNA size marker

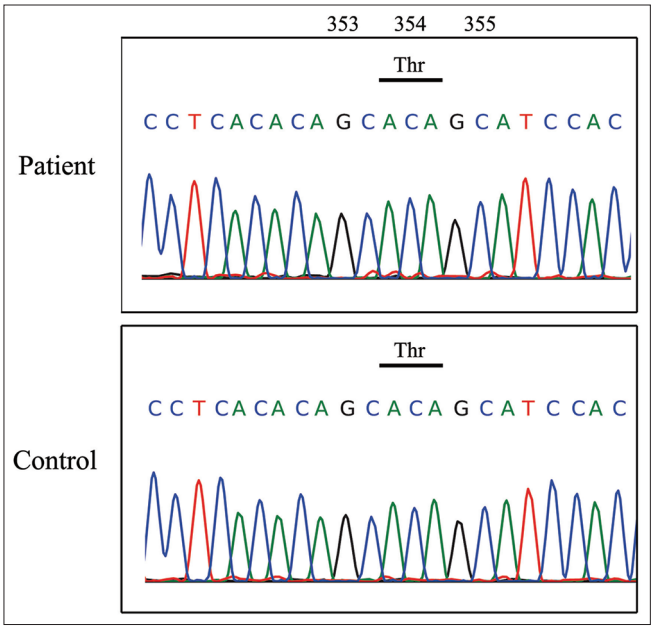

Figure 2: Electropherograms of T354P mutation of the sodium iodide symporter gene in a sample dyshormonogenetic congenital hypothyroidism patient and a control neonate

Financial support and sponsorship

This study was supported in part by a research grant from Isfahan University of Medical Sciences, Isfahan, Iran.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Klett M. Epidemiology of congenital hypothyroidism. Exp Clin Endocrinol Diabetes 1997;105 Suppl 4:19-23.
2. Harris KB, Pass KA. Increase in congenital hypothyroidism in New York State and in the United States. Mol Genet Metab 2007;91:268-77.
3. Hashemipour M, Amini M, Iranpour R, Sadri GH, Javaheri N, Haghighi S, *et al.* Prevalence of congenital hypothyroidism in Isfahan, Iran: Results of a survey on 20,000 neonates. Horm Res 2004;62:79-83.
4. Rastogi MV, LaFranchi SH. Congenital hypothyroidism. Orphanet J Rare Dis 2010;5:17.
5. Park SM, Chatterjee VK. Genetics of congenital hypothyroidism. J Med Genet 2005;42:379-89.
6. Foley T. Congrnital hypothroidism. In: Braverman L, Utiger R, editors. Werner and Ingbar's the Thyroid: A Fundamental and Clinical Text. 7th ed. Philadelphia: Lippincott Williams and Wilkins; 1996. p. 988-94.
7. Toublanc JE. Comparison of epidemiological data on congenital hypothyroidism in Europe with those of other parts in the world. Horm Res 1992;38:230-5.
8. Ilicki A, Larsson A, Karlsson FA. Circulating thyroid antibodies in congenital hypothyroidism. Acta Paediatr Scand 1991;80:805-11.
9. Azizi F, Sheikholeslam R, Hedayati M, Mirmiran P, Malekafzali H, Kimiagar M, *et al.* Sustainable control of iodinedeficiency in Iran: Beneficial results of the implementation of the mandatory law on salt iodization. J Endocrinol Invest 2002;25:409-13.
10. Hashemipoor M, Amini M, Gheisari A, Sharifei S, Iranpour R, Aminorroaya A. Comparison of urinary iodine excretion in neonates and their mothers in Isfahan, Iran. Endocr Pract 2002;8:347-50.
11. Van Vliet G. Development of the thyroid gland: Lessons from congenitally hypothyroid mice and men. Clin Genet 2003;63:445-55.
12. Clifton-Bligh RJ, Wentworth JM, Heinz P, Crisp MS, John R, Lazarus JH, *et al.* Mutation of the gene encoding human TTF-2 associated with thyroid agenesis, cleft palate and choanal atresia. Nat Genet 1998;19:399-401.
13. Congdon T, Nguyen LQ, Nogueira CR, Habiby RL, Medeiros-Neto G, Kopp P. A novel mutation (Q40P) in PAX8 associated with congenital hypothyroidism and thyroid hypoplasia: Evidence for phenotypic variability in mother and child. J Clin Endocrinol Metab 2001;86:3962-7.
14. Meeus L, Gilbert B, Rydlewski C, Parma J, Roussie AL, Abramowicz M, *et al.* Characterization of a novel loss of function mutation of PAX8 in a familial case of congenital hypothyroidism with in-place, normal-sized thyroid. J Clin Endocrinol Metab 2004;89:4285-91.
15. Borck G, Topaloglu AK, Korsch E, Martiné U, Wildhardt G, Onenli-Mungan N, *et al.* Four new cases of congenital secondary hypothyroidism due to a splice site mutation in the thyrotropin-beta gene: Phenotypic variability and founder effect. J Clin Endocrinol Metab 2004;89:4136-41.
16. Smanik PA, Ryu KY, Theil KS, Mazzaferri EL, Jhiang SM. Expression, exon-intron organization, and chromosome mapping of the human sodium iodide symporter. Endocrinology 1997;138:3555-8.
17. Spitzweg C, Morris JC. Genetics and phenomics of hypothyroidism and goiter due to NIS mutations. Mol Cell Endocrinol 2010;322:56-63.
18. De La Vieja A, Dohan O, Levy O, Carrasco N. Molecular analysis of the sodium/iodide symporter: Impact on thyroid and extrathyroid pathophysiology. Physiol Rev 2000;80:1083-105.
19. Mostofizade N, Nikpour P, Javanmard SH, Emadi-Baygi M, Miranzadeh-Mahabadi H, Hovsepian S, *et al.* The G395R mutation of the Sodium/Iodide symporter (NIS) gene in patients with dyshormonogenetic congenital hypothyroidism. Int J Prev Med 2013;4:57-62.
20. Fujiwara H, Tatsumi K, Miki K, Harada T, Miyai K, Takai S, *et al.* Congenital hypothyroidism caused by a mutation in the Na+/I- symporter. Nat Genet 1997;16:124-5.
21. Fujiwara H, Tatsumi K, Miki K, Harada T, Okada S, Nose O, *et al.* Recurrent T354P mutation of the Na+/I- symporter in patients with iodide transport defect. J Clin Endocrinol Metab 1998;83:2940-3.
22. Kosugi S, Sato Y, Matsuda A, Ohyama Y, Fujieda K, Inomata H, *et al.* High prevalence of T354P sodium/iodide symporter gene mutation in Japanese patients with iodide transport defect who have heterogeneous clinical pictures. J Clin Endocrinol Metab 1998;83:4123-9.
23. Spitzweg C, Morris JC. The sodium iodide symporter: Its pathophysiological and therapeutic implications. Clin Endocrinol (Oxf) 2002;57:559-74.