

## The G395R Mutation of the Sodium/Iodide Symporter (*NIS*) Gene in Patients with Dyshormonogenetic Congenital Hypothyroidism

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### ABSTRACT

**Background:** Considering the high prevalence of congenital hypothyroidism (CH) in Isfahan and its different etiologies in comparison with other countries, the high rate of parental consanguinity, and the role of *NIS* gene in permanent CH due to dyshormonogenesis, the aim of this study was to investigate the G395R mutation of the *NIS* gene in patients with permanent CH due to dyshormonogenesis

**Methods:** In this case-control study, patients diagnosed with permanent CH due to dyshormonogenesis during CH screening program were selected. Venous blood sample was obtained to determine the G395R mutations of *NIS* gene using polymerase chain reaction (PCR) sequencing method.

**Results:** In this study, 35 CH patients with permanent CH due to dyshormonogenesis and 35 neonates with normal screening results as a control group were studied. We did not find any changes of the mentioned mutation of *NIS* gene in the patients' group.

**Conclusion:** Considering the findings of the current study, it seems that further studies with larger sample size and with consideration of other gene mutations such as pendrin and thyroglobulin are needed for more accurate conclusion.

**Keywords:** Congenital hypothyroidism, dyshormonogenesis, G395R, mutation, sodium/iodide symporter (*NIS*) gene

### INTRODUCTION

Congenital hypothyroidism (CH), with a prevalence rate of 1 in 3000-4000 live births, is considered as the most common endocrine disorder in neonates.<sup>[1]</sup> Early diagnosis and treatment of neonates with CH is crucial for their neurological development, otherwise it results in irreversible deleterious developmental effect and mental retardation.<sup>[2]</sup>

In 85%-90% of permanent CH cases, the etiology is thyroid dysgenesis (TD). The transcription factor genes, *TITF-1*, *FOXE-1*, and *PAX-8* have an important role in the pathogenesis of TD.<sup>[3]</sup>

Thyroid dyshormonogenesis is responsible for 10%-15% of permanent CH cases. Patients with thyroid dyshormonogenesis have inborn errors of thyroid hormonogenesis. It shows autosomal recessive inheritance, consistent with a single gene mutation in genes such as *TPO*, *Tg*, *TSH-R* *THOX*, *Pendrin*, and *NIS*.<sup>[4]</sup>

Iodide transport defect (ITD) is one of the causes of thyroid dyshormonogenesis. It is an uncommon disorder which is mediated by *NIS* mutations. ITD cases are represented by hypothyroidism and goiter due to the absence of active transport of iodide into the thyroid gland.<sup>[5]</sup>

After the molecular characterization of *NIS* gene in 1996, 13 mutations in this symporter that causes ITD have been identified so far.<sup>[6-15]</sup>

Though the clinical presentation of patients with ITD due to different mutations of *NIS* gene has been described properly, only T354P, G395R, Q267E, and G543E mutations have been characterized at the molecular level.<sup>[16,17]</sup>

G395R was first described by Kosugi *et al.* in members of a family with ITD. The molecular studies of G395R mutation have indicated that the presence of a small and uncharged aa residue at position 395 is required for *NIS* activity and defect in this position results in ITD.<sup>[17,18]</sup>

CH is considered as a common problem in our region with a prevalence rate of 1/370 live births.<sup>[19]</sup> Moreover, etiologic studies in Isfahan show that in most of these CH patients, the etiology of CH is thyroid dyshormonogenesis which is not similar to that reported worldwide.<sup>[20]</sup> Thus, it seems that the identification of important genes involved in thyroid hormonogenesis including *NIS* mutations as underlying cause of ITD would provide us a basis for a molecular diagnosis and genetic counseling in patients with CH due to dyshormonogenesis. The aim of the present study was to determine the G395R mutation of *NIS* gene in this group of CH patients in Isfahan.

## METHODS

In this case-control study, dyshormonogenetic CH patients diagnosed and followed up during CH screening program (2002-2011) in Isfahan Endocrine and Metabolism Research Center were enrolled. CH screening program in Isfahan was initiated in 2002 and continued until 2005 when the nationwide CH screening program was implemented.

Newborns with abnormal screening results were re-examined, and those with abnormal T4 and thyroid stimulating hormone (TSH) levels on their second measurement (TSH > 10 mIU/l and T4 < 6.5 µg/dl) were diagnosed as CH patients and received treatment and regular follow-up.

Hypothyroid neonates underwent treatment at a dose of 10-15 µg/kg/day as soon as the diagnosis was confirmed. The TSH and T4 levels were monitored during the follow-up. Permanent and transient cases of CH were determined at the age of 3 years by measuring TSH and T4 concentrations 4 weeks after withdrawal of L-T4 therapy. Patients with elevated TSH levels (TSH > 10 mIU/l) and decreased T4 levels (T4 < 6.5 µg/dl) were considered as permanent CH sufferers. The etiology of CH was determined by thyroid scan and/or ultrasound before treatment in neonatal period or at the age of 3 years after confirming the permanency of CH. Patients with thyroid gland of normal size were considered to have dyshormonogenesis.<sup>[20]</sup>

The Medical Ethics Committee of Isfahan University of Medical Sciences and the Institutional Review Board of Isfahan Endocrine and Metabolism Research Center approved the study protocol.

Patients were selected by random sampling method and a group of age- and sex-matched children with normal screening results were selected as the control group and recalled for participation in the study. Written informed consent was obtained from parents of all selected CH patients and control group.

All selected children in case and control groups were examined by a pediatrician (NM), and the demographic characteristics and screening findings regarding the level of TSH and T4, and parental consanguinity were recorded using a questionnaire.

Venous blood samples measuring 4 ml were collected in ethylenediaminetetraacetic acid (EDTA) tubes from the studied population.

### Laboratory measurements

Serum T4 and TSH were measured by radioimmunoassay (RIA) and immunoradiometric assay (IRMA) methods, respectively.

### Genomic DNA extraction

Peripheral blood samples were collected in EDTA-anticoagulated tubes, and then genomic

DNA was extracted from the whole blood using the Diatom DNA Prep 100 kit (Isogen Laboratory, Moscow, Russia), according to the manufacturer's instructions. DNA quantity and quality were assessed by UV absorption at 260 and 280 nm and by agarose gel electrophoresis.

### Polymerase chain reaction amplification

Polymerase chain reaction (PCR) was used to amplify genomic DNA fragments of *NIS* gene containing the G395R mutation. Cycling conditions were at 95°C for 5 min (one cycle); at 95°C for 40 sec, at 55°C for 40 sec, at 72°C for 60 sec (for 35 cycles); and final extension at 72°C for 10 min using a pair of primers annealing at flanking introns. PCR primers were designed using Gene Runner software (version 3.02; Hastings software Inc.) according to human *NIS* genomic sequence (NCBI Reference Sequence: NG\_012930.1). The sequences of primers were: hNIS-Forward: TATCTCCTTCACCTTTGCAG and NIS-Reverse: ACTGACACATGTGACCTCTCTG. PCR reaction was performed in a 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<sub>2</sub>), and 1.25 Units of DFS-Taq polymerase (Macrogen, Seoul, South Korea). The identity of PCR products was further confirmed on a 1.5% agarose gel, stained with ethidium bromide, and visualized under the ultraviolet light.

### Direct DNA sequencing

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 the BioEdit (version 7.1.3; Hall, T. A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucl. Acids. Symp. Ser. 41:95-98), Sequence scanner (version 1.0; Applied Biosystems Co.), and Nucleotide BLAST programs.<sup>[21-23]</sup>

## RESULTS

In this study, 35 CH patients with permanent CH due to dysmorphogenesis and 35 neonates with normal screening results as a control group were assessed. Demographic characteristics of the studied population are presented in Table 1.

**Table 1:** Demographic characteristics of patients with permanent CH due to dysmorphogenesis and the control group

	Dysmorphogenetic CH patients	Control group	P value
Age (months)	64.6±23.7	69.3±27.2	NS
Sex (male/female)	19/16	17/18	NS
Parental consanguinity	62%	39%	<0.05
TSH (mIU/l)			
Primary	47.3±46.1	4.5±2.7	<0.05
After treatment interruption	36.5±30.7	-	-
T4 (µg/dl)			
Primary	6.2±3.5	11.6±3.9	<0.05
After treatment interruption	7.1±2.9	-	-

NS: Not significant

The quantity and quality of extracted DNA assessed by UV absorption and by agarose gel electrophoresis is presented in Figure 1. Figure 2 shows the specific amplification of a 445 bp amplicon of *NIS* gene using specific primers. Electropherograms of G395R mutation of *NIS* gene in a sample dysmorphogenetic CH patient and a control neonate are presented in Figure 3. Comparison of nucleotide sequences of all amplified PCR products with human *NIS* genomic sequence by BLAST online software showed no polymorphism in our population [Figure 4].

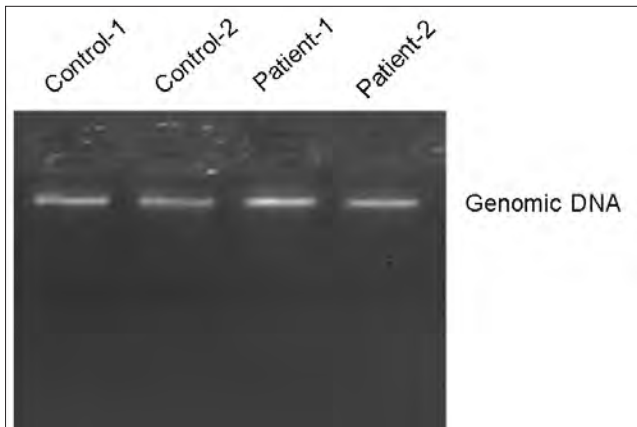
Overall, we did not find any G395R mutation of *NIS* gene in the studied population.

## DISCUSSION

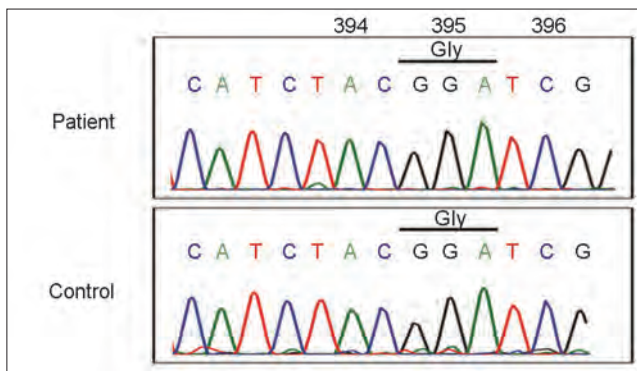
In this study, the presence of G395R mutation of *NIS* gene in CH patients with thyroid dysmorphogenesis was evaluated and the findings indicated that there was not any G395R mutation of *NIS* gene among the studied population.

Many human and animal studies have suggested the important role of *NIS* gene in the synthesis of thyroid hormone and the etiology of CH.<sup>[24-26]</sup> *NIS* mutations cause ITD, a disorder that if not diagnosed and treated during the early infancy period results in dysmorphogenesis.<sup>[27]</sup>





**Figure 1:** The quality of extracted DNA assessed by agarose gel electrophoresis

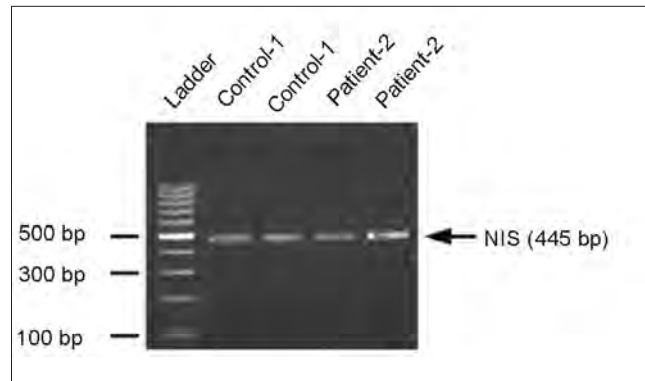


**Figure 3:** Electropherograms of G395R mutation of *NIS* gene in a sample dysmorphogenetic CH patient and a control neonate

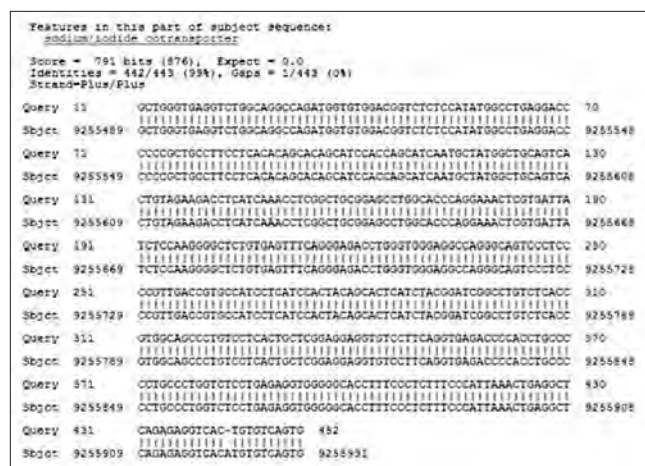
*NIS* is a specialized plasma membrane glycoprotein that mediates active iodide transport. It is located at the basolateral membrane of thyrocytes and has an important role in active iodide trapping from the bloodstream in thyrocytes.<sup>[28]</sup>

Human *NIS* gene was cloned for the first time in 1996. It is located on chromosome 19 and consists of 15 exons, and encodes a protein with 643 amino acids.<sup>[29]</sup> The G395R *NIS* protein is synthesized and properly targeted to the membrane.<sup>[28]</sup>

G395R, as an ITD causing loss of function mutation, was first described by Kosugi *et al.* in the members of a family with a history of ITD and high rate of parental consanguinity in 1999 in Canada. Past medical history of the mentioned family revealed that nine children from the family had been diagnosed with CH during neonatal CH screening program. After neonatal period, seven of nine CH patients were diagnosed with ITD.<sup>[17]</sup>



**Figure 2:** The amplification of genomic DNA fragments of *NIS* gene using specific primers



**Figure 4:** Comparison of nucleotide sequences of the amplified PCR products with human *NIS* genomic sequence by BLAST online software

After the first description of G395R mutation, Dohan and colleagues investigated the molecular basis of the mutation. According to their findings, the presence of an uncharged amino acid residue with a small side chain at position 395 is a requirement for *NIS* function, suggesting that glycine 395 is located in a tightly packed membrane-embedded region of *NIS*. The presence of charge or of a long side chain at position 395 results in decreased turnover rate of the transporter, without affecting its ion binding affinity.<sup>[18]</sup>

Patients with G395R mutation have some specific characteristics, including that in contrast to other mutations, goiter was not developed in them and most of them are severely hypothyroid which is detected during the neonatal period. This was the reason why this mutation was studied among CH patients.<sup>[30]</sup>

In the current study, we did not find any

G395R mutation of *NIS* gene among the studied population. The obtained result could be explained as follows.

ITD is a rare form of dyshormonogenetic CH, so it is possible that it is not the etiologic factor of CH among our studied patients. Moreover, we studied CH patients with thyroid dyshormonogenesis without determining the etiology of dyshormonogenesis. However, ITD is one of the uncommon causes of it.

The diagnostic criteria for ITD include a variable degree of CH and goiter, low or absent radioiodide uptake (RIUT) by the thyroid and other *NIS*-expressing organs (such as the salivary glands and gastric mucosa), and a low iodide saliva-to-plasma (S/P) ratio.<sup>[31]</sup>

Clinical examination of the studied patients indicated that none of them had goiter. It may be due to early diagnosis and treatment of the patients. Evidences showed that goiter may represent in these patients later, not in the first years of life.<sup>[30]</sup>

Radioiodine uptake was not measured in our studied population because most of the parents of CH patients did not allow performing that test and the etiology of CH was determined mostly by ultrasonography and not thyroid scintigraphy.

There were no facilities to measure the iodide saliva-to-plasma (S/P) ratio in our current settings.

So, it seems that by studying larger sample of CH patients with accurate determination of the cause of dyshormonogenesis, such mutations in *NIS* gene may be found in the patients.

It is also suggested that other genetic disorders may be more prevalent than G395R mutation of *NIS* gene in CH patients with dyshormonogenesis, such as thyroglobulin gene. The role of other genes such as *DUOX2* and *TPO* was investigated in our region.<sup>[32,33]</sup>

## CONCLUSION

In conclusion, it seems that G395R mutation of *NIS* gene does not play a major role in the etiology of thyroid dyshormonogenesis among patients with CH in Isfahan, but keeping in mind the explanations given, it is necessary to perform more studies with a larger sample size, and mutation detection of other genes that have major effect on thyroid dyshormonogenesis and have not been studied yet in our population needs to be done.

In addition, determining CH patients with ITD would be more helpful in this regard.

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