Mutation screening of the sodium iodide symporter gene in a cohort of 105 China patients with congenital hypothyroidism

Análise de mutações no gene simportador sódio/iodeto em coorte de 105 pacientes chineses com hipotireoidismo congênito

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ABSTRACT

Objective: Dyshormonogenetic congenital hypothyroidism (CH) was reported to be associated with a mutation in the sodium iodide symporter (NIS) gene. The present study was undertaken in the Guangxi Zhuang Autonomous Region of China, to determine the nature and frequency of NIS gene mutations among patients with CH due to dyshormonogenesis. **Subjects and methods:** Blood samples were collected from 105 dyshormonogenetic CH patients in Guangxi Zhuang Autonomous Region, China, and genomic DNA was extracted from peripheral blood leukocytes. All exons of the NIS gene together with their exon-intron boundaries were screened by next-generation sequencing. **Results:** Two silent variations (T221T and T557T) and one missense variation (M435L), as well as two polymorphisms (rs200587561 and rs117626343) were found. **Conclusions:** Our results indicate that the NIS mutation rate is very low in the Guangxi Zhuang Autonomous Region, China, and it is necessary to study mutations of other genes that have major effects on thyroid dyshormonogenesis and have not as yet been studied in this population. Arq Bras Endocrinol Metab. 2014;58(8):828-32

Keywords

Congenital hypothyroidism; sodium iodide symporter gene; gene mutations; China; next-generation sequencing

RESUMO

Objetivo: O hipotireoidismo congênito disormonogenético (CH) foi relatado como associado a uma mutação no gene simportador sódio/iodeto (NIS). O presente estudo foi feito na região autônoma de Guangxi Zhuang na China para se determinar a natureza e a frequência das mutações no gene NIS entre pacientes com CH causado por disormonogênese. **Sujeitos e métodos:** Amostras de sangue foram coletadas de 105 pacientes com CH disormonogenéticos e o DNA genômico foi extraído de leucócitos do sangue periférico. Todos os éxons do gene NIS, junto com seus limites éxon-íntron, foram analisados por sequenciamento de nova geração. **Resultados:** Foram encontradas duas variações silenciosas (T221T e T557T) e uma variação missense (M435L), assim como dois polimorfismos (rs200587561 e rs117626343). **Conclusões:** Nossos resultados indicam que a taxa de mutação em NIS é muito baixa na região de Guangxi Zhuang. É necessário estudar mutações de outros genes que tenham efeitos maiores na disormonogênese da tiroide e que ainda não tenham sido estudados nesta população. Arq Bras Endocrinol Metab. 2014;58(8):828-32

Descritores

Hipotireoidismo congênito; gene simportador sódio/iodeto; mutações genéticas; China; sequenciamento de nova geração

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INTRODUCTION

Congenital hypothyroidism (CH) is a common endocrine disorder in newborns, with prevalence ranging from 1:2000 to 1:4000 (1,2). The etiology of congenital hypothyroidism is heterogeneous. Eighty to eighty-five percent of cases are caused by disorders of thyroid gland development (3), which has been linked to mutations in TSHR, PAX8, NKX2.1, NKX2.5 and FOXE1 genes (3); the remaining 15-20% of cases are due to the abnormalities in thyroid hormone synthesis (dyshormonogenesis) (4).

Defects in the human NIS gene are reported to be one of the causes of CH due to dyshormonogenesis (5). NIS is a specialized plasma membrane glycoprotein that has an important role in active trapping of iodine from the bloodstream into thyrocytes, the key step in the biosynthesis of the iodine-containing thyroid hormones T_3 and T_4 (6). NIS gene mutations can cause iodide transport defects (ITD) (7-9), a disorder that if not diagnosed and treated during the early infancy period would result in CH due to dyshormonogenesis. Up to now, the mutational spectrum of the NIS and the genotype-phenotype relationships has not yet been fully established, and there is also no report of human NIS mutations in the Chinese population. Here, we report an investigation on the prevalence of inactivating NIS mutations in the Guangxi Zhuang Autonomous Region, China. The primary objective was to screen for the presence of mutations in NIS gene among patients with dyshormonogenetic CH in the Guangxi Zhuang Autonomous Region.

SUBJECTS AND METHODS

Subjects

We enrolled 105 patients with dyshormonogenetic CH, who were identified through newborn screening among 353,000 newborns in the Guangxi Zhuang Autonomous Region, China, from October 2010 to June 2013. All of the subjects underwent neonatal screening using filter paper for CH at 72h after birth when blood samples were collected from the heel and TSH levels were measured by enzyme-linked immunosorbent assay. Subjects with increased TSH (TSH ≥ 10 uU/mL) levels observed during neonatal screening were recalled for further evaluation. Serum TSH and FT4 were determined by electrochemiluminescence assay. Diagnosis of CH is based on elevated TSH levels (normal range 0.27-4.2 µIU/mL) and decreased FT4 levels (normal range 12-22 pmol/L). The etiology of CH was determined by thyroid scan and/or ultrasound before treatment in the neonatal period or at the age of 3 years after confirming the permanency of CH. Patients with normal size or enlarged thyroid gland were considered to have dyshormonogenesis. The study was approved by the local Medical Ethics Committee. Informed consent was obtained from the parents of the patients.

Mutation detection

Peripheral venous blood samples were collected from the patients. Genomic DNA was extracted from peripheral blood leukocytes using QIAamp DNA Blood Mini Kit (Qiagen, Germany) according to the manufacturer's protocol. Exons 1 to 15 of NIS with their flanking intronic regions were amplified in a 50 µL reaction using the primers shown in table 1. The 50 µL of PCR reaction mixture containing 100-250 ng of genomic DNA, 1× Taq Buffer with 50 mM of KCl, 2.5 mM of MgCl, 200 µM of dNTP, 1unit of Taq DNA polymerase (Fermentas, USA), and 20 pmol of forward and reverse primers were prepared. Thirty cycles of amplification were carried out with a standard PCR protocol. PCR products were purified using QIA quick PCR purification kit (Qiagen, Germany) following the manufacturer's instructions. The purified PCR products were sequenced using Illumina MiSeq next generation sequencing instrument (Illumina, America). A control group of 600 healthy subjects without thyroid disease was also screened for the same mutations. All control subjects had normal FT4 (12-22 pmol/L) and TSH levels (0.27-4.2 µIU/mL).

Functional prediction of mutation

The potential functional impact of variations were predicted using both sorting intolerant from tolerant (SIFT) and Polymorphism Phenotyping 2 (PolyPhen-2), to predict the degree of (possible) impact of an amino acid substitution on the structure and function of a human protein. The functional impact of the mutation was depicted as "tolerated" or "damaging" for SIFT and predicted as "benign", "possibly damaging", "probably damaging", or "unknown" for PolyPhen.

RESULTS

We identified five variations in the NIS gene in 105 dyshormonogenetic CH patients (Table 2). The five varia-

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tions include two silent variations (T221T and T557T), one missense variation (M435L) and two polymorphisms (rs200587561 and rs117626343). The unreported variations in the NIS gene were tested against DNA samples from 600 healthy control subjects.

The NIS protein sequences of different species, including Homo sapiens, Mus musculus, Rattus norvegicus, Bos taurus, Danio rerio and Sus scrofa were obtained from the National Center for Biotechnology Information (NCBI) website. Using DNAMAN software, we observed multiple sequence alignment of the NIS gene family of various species. We found that codon 435 where the missense mutation (p.M435L) was identified, was located in a highly conserved region of NIS (Figure 1).

The potential functional impact of M435L was predicted using both SIFT and PolyPhen-2 and the prediction result was depicted as "tolerated" for SIFT and "benign" for PolyPhen-2 (Figure 2).

Table 1. List of NIS primers

Exon	Forward primer	Reverse primer
E1	5'-GGCAGGACAGACAGACAGCAGG-3'	5'-GGAAACCCAAACAGAGAGGCACG-3'
E2-4	5'-GCCCACCTAGAGAGCAGACCAG-3'	5'-CAATCTCCACGCACCGCAACC-3'
E5	5'-AGGCATAGAAGGGCATCAGTCC-3'	5'-GTGCAGTAGTGTGATCTCAGCTTG-3'
E6-7	5'-CAAAACCCACTCCAAATGTCCC-3'	5'-ATCTCCCTTCATCGCAACCTCC-3'
E8-10	5'-CTCTGAGCCCTGTCCGTCTTG-3'	5'-GGAGATTGAGGTGGAAGGATG-3'
E11-12	5'-TCAAGCAACCACCCGCCTCAG-3'	5'-GGAGATTGAGGTGGAAGGATG-3'
E13	5'-GTAATGTGCCCTTTGGCTGT-3'	5'-GCTCAGGAGTTCAAGGTTGC-3'
E14	5'-TACTGGGCAACTCTAAGCAATC-3'	5'-ACATCAATACCCTAACAGCACAC-3'
E15	5'-AGCAGAGAAAGGAGGGAGGAAC-3'	5'-ATTGAGAAAAACGGTAGGCTGG-3'

Table 2. Previously reported and novel variations identified in the present study

Exon	NIS gene variants	Type	Reference	Frequency of minor alleles in dyshormonogenetic CH patients (210 alleles)	Frequency of minor alleles in healthy control subjects (1200 alleles)
7	C310C	SNP	rs200587561	0.0048	NA
10	L408L	SNP	rs117626343	0.0048	NA
5	T221T	Silent variation	Not reported	0.0048	0.0050
14	T557T	Silent variation	Not reported	0.0048	0.0058
11	M435L	Missense variation	Not reported	0.0142	0.00

NA: not analyzed in healthy controls.

Figure 1. Multiple sequence alignment of sodium iodide symporter (NIS) genes from Homo sapiens, Mus musculus, Rattus norvegicus, Danio rerio, Sus scrofa and Bos taurus. The arrow indicates that the glycine 435 residue is located within a highly conserved region.

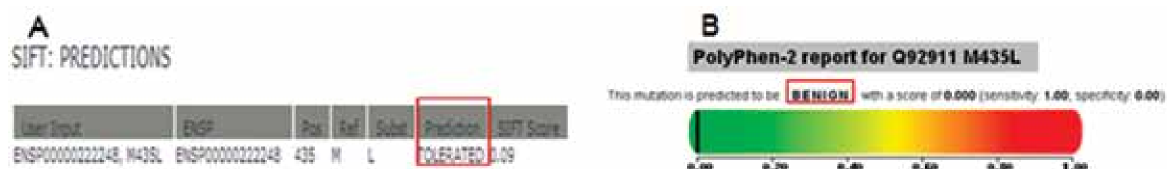

Figure 2. Functional prediction of the M435L variation. (**A**) The functional impact of the variation was depicted as "tolerated" for SIFT. (**B**) The functional impact of the variation was depicted as "benign" for PolyPhen-2.

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DISCUSSION

Human NIS gene was cloned for the first time in 1996. It is located on chromosome 19p12-13.2 and consists of 15 exons, and encodes a protein of 643 amino acids with a molecular mass of approximately 70-90 kDa (10). The secondary structure of NIS is an intrinsic membrane protein with 13 transmembrane segments, an extracellular amino-terminus and an intracellular carboxyl-terminus (11,12). Up to now, 15 mutations in this symporter gene that causes ITD have been identified: -54C >T, V59E, G93R, R124H, Δ142-323, Q267E, C272X, Δ287-288, T354P, G395R, Δ439-443, frameshift 515X, Δ439-443, Y531X, and G543E (13-15). Some of these ITD mutants, including V59E, G93R, Δ439-443, R124H, Q267E, T354P, G395R, and G543E, have been studied in detail and have provided key mechanistic information on NIS (15-22).

Because NIS mutations are inherited in an autosomal recessive manner, heterozygous individuals do not present with the phenotype. NIS gene defects can be detected only when both alleles are mutated. Furthermore, under the conditions of high iodine intake, when full preservation of iodine concentrating function is not required to achieve normal thyroid hormone synthesis, mutations causing impairments of function may not be detected even in the homozygous patients. For these reasons, the actual prevalence of NIS gene mutations may be much higher than that reported.

To confirm our hypothesis and to investigate the frequency of NIS gene mutations in China, we screened NIS mutations in a cohort of 105 dyshormonogenetic CH patients from the Guangxi Zhuang Autonomous Region in China. In all, two silent variations (T221T and T557T) and one missense variation (M435L), as well as two polymorphisms (rs200587561 and rs117626343) were found. The mutated Met435 residue lies in the midst of the putative eleventh transmembrane segment of the NIS protein, although it is conserved among species, in addition, the M435L variation was not found in 600 control individuals (Table 2), yet the potential functional impact of the variation was predicted to be "benign" both by SIFT and PolyPhen-2.

In the current study, we did not find any inactivating mutation of NIS gene among the studied population. The following two possibilities could account for the obtained results: 1) the NIS mutation rate is very low in our CH patients and 2) we studied CH patients with thyroid dyshormonogenesis without selection based on etiology, as ITD is a rare form of dyshormonogenetic CH. The identification criteria for ITD includes low or absent radioiodide uptake (RIUT) by the thyroid and several extrathyroidal tissues [such as the salivary glands, lactating mammary gland and gastric mucosa (23-27)], and a low iodide saliva-to-plasma (S/P) ratio (28).

Understandably, most of the parents of CH patients did not allow the radioiodine uptake test to be undertaken and the etiology of CH was determined mostly by ultrasonography and not by thyroid scintigraphy, the radioiodine uptake test was not performed in our studied population.

In addition, there was no facility to measure the iodide saliva-to-plasma (S/P) ratio in our current settings.

Taken together, by studying a large sample of patients with accurate determination of the etiology of thyroid dyshormonogenesis, mutations in NIS gene may be found in the patients.

Interestingly, we also found that some of the patients had normal sized thyroid gland as determined by clinical examination, but goiter could present in them later although not in the first years of life. This may be due to early diagnosis and treatment of these patients.

In conclusion, our results indicate that mutations of NIS gene do not play a major role in the etiology of thyroid dyshormonogenesis among patients with CH in the Guangxi Zhuang automous region of China. Future studies may include mutation detection of other genes that have major effects on thyroid dyshormonogenesis and have not as yet been studied in our population.

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