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Study on DEHAL1 Mutations in Patients with Congenital Hypothyroidism and Thyroid Goiter

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Abstract: The objective of this research is to study the types and characteristics of *DEHAL1* gene mutation in patients with congenital hypothyroidism (CH) and thyroid goiter from Shandong Province, which can provide some evidence for gene diagnosis of CH. 47 cases of patients who were diagnosed as CH combined with thyroid goiter by neonatal screening and 100 normal controls were selected as subjects and their genome DNA were extracted. All the exons were amplified by polymerase chain reaction (PCR) and PCR products were sequenced by direct sequencing (Sanger sequencing). DNA sequencing results were compared to the *DEHAL1* gene reference sequence to see whether there was mutation, and $\chi 2$ test was used on the gene frequency of discovered Single Nucleotide Polymorphisms (SNP). The results showed that no *DEHAL1* gene mutation was found in 60 cases of CH with thyroid goiter patients and 100 normal controls, however, two SNPs were found(rs672766, IVS3+129C>T; rs2076292, IVS3+142C>T) in intron region. There was no significant difference between the SNP rate in CH patients and normal controls ($P > 0.05$). It can be concluded that *DEHAL1* gene mutation rate is very low which may not be the main factor leading to the congenital hypothyroidism (CH) with thyroid goiter in Shandong Province, China.

Keywords: Congenital hypothyroidism; *DEHAL1*; Thyroid goiter; Mutation; Child

Introduction

Congenital hypothyroidism is a common endocrine and metabolic disease in children, occurring in 1 of 3500 newborns [1] and the number of girls is two times as much as the boy[2].CH can lead to delayed growth and mental retardation, which is commonly known as "cretinism" [3]. Congenital hypothyroidism can be divided into two major types according to its pathogenesis. 80%-85% of CH is caused by defective thyroid glands, such as athyreosis, hypoplastic or ectopic gland[4],which is closely related to the gene encoding thyroid transcription factor, such as *TSHR*[5], *TTF1*[6],*PAX8*[7],*NKX2.1*[8] and *FOXE1*[9] and so on. 15%-20% of CH patients with thyroid dyshormonogenesis combined with thyroid goiter [1] is inherited as an autosomal recessive trait[10]. Genetic defects of enzymes in the thyroid hormone synthesis pathway, such as *DEHAL1*[11], thyroglobulin (*TG)*[12], thyroid peroxidase (*TPO)*[13], dual oxidase 2 maturation factor (*DUOXA2)*[14], dual oxidase 2 (*DUOX2)*[15], sodium-iodide symporter (*NIS)*[16] and *Pendred syndrom* (*PDS)*[17], can cause thyroid dyshormonogenesis.

Patients with iodotyrosine dehalogenase deficiency (ITDD) had been described in the 1950s on the basis of chromatographic studies with radioactive-labeled compounds and measurement of the enzymatic activity in goitrous thyroid gland[18]. In 2002, the use of Serial Analysis of Gene Expression (SAGE)[19] applied to human thyroid tissue, allows the cloning of *DEHAL1*

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gene[20]. *DEHAL1* is organized in 6 exons (NM_001164694), encoding 293 amino acid, spanning over 35 kb on human chromosome 6p 24. *DEHAL1* is mainly expressed in thyroid, in addition, it is also present in the liver, kidney, and colon at low levels. *DEHAL1* gene encodes iodotyrosine deiodinase which is responsible for the deiodination of MIT and DIT ensuring iodine recycling for thyroid hormone biosynthesis[21]. In 2008, the first *DEHAL1* mutations were reported in three different consanguineous families, 3 homozygous mutations, two missense (R101W,I116T) and one inframe-deletion of three base pairs(F105-I106L)[22].

Then a mutation(c.658G>A,p.Ala220Thr) was described in a consanguineous Moroccan family. Surprisingly, this mutation was not only found in a homozygous patient but also found in a 14-year-old boy. Research suggests that the phenotypic variation in patients is difficult to explain. On one hand, it may be caused by environment factors. On the other hand, there may be another mutation in the subject. Currently, research on *DEHAL1* gene is involved little in our country. 60 cases of patients with CH and thyroid goiter diagnosed by neonatal screening and 100 normal controls in Shandong province were selected as subjects in our study. Their genome DNA were extracted and all the exons of *DEHAL1* were amplified by Polymerase Chain Reaction (PCR). The types and characteristics of *DEHAL1* gene were studied combining with the sequencing data of PCR products in order to provide some evidence for gene diagnosis of CH.

Materials and Methods

Patients

60 cases of patients who were diagnosed as CH combined with thyroid goiter by neonatal screening and 100 normal controls came from Women and Children Hospital of Qingdao. To be included in the study, patients were required to meet the following features at diagnosis: (1) TSH level was over 10mIU/L during the confirmatory test. (2) TSH increased, FT_4 decreased, FT_3 normal or decreased during the test for thyroid function [thyroid stimulating hormone (TSH), free thyroid hormone (FT_4), free three iodine thyroid acid (FT_3)]. (3)The normal thyroid gland combined with thyroid goiter after lining 99mTc thyroid scanning or B ultrasound examination. (4) Subjects come from 60 different consanguineous families of Shandong province and had no other congenital diseases after making physical test and B ultrasound examination. 100 cases healthy individuals were enrolled. This study was approved by the medical ethics committee of the Affiliated Hospital of Qingdao University.

Methods: DNA sequencing

Peripheral blood DNA from patients was extracted by the proteinase K method. The complete coding sequence of the *DEHAL1* gene, including splice sites and flanking intronic regions of each exon, was amplified by primers (Table.1). PCR products were separated by 15g/L agarose gel and scanned by UVP gel imaging instrument. PCR products whose band are single and bright can be sequenced.

Table 1 The primer sequence of all exons in *DEHAL1* gene

exon	forward primer(5'→3')	reverse primer(5'→3')	amplified products length(bp)
1	ATTCTCCACTCCTGTCTC	AATAGAGGTTGATGTTGAA	379
2	CAAGGGATCATTTAGTTTG	CTCAGCATTTGGGTAAGA	379
3	TGCTTGGACTACAGGGAT	ATGGCAATACAGGAGTGAG	415
4	GACCTGCCCTTGTATCTT	ATTTCCAAATGTCCCTGA	489
5	TGGCATTGATTTCCTTCC	CACCACCTCTAAACCTGACC	366
6	CGATGCCATTACTTGAGC	CCTGACACCTGGAGAAAGA	446

Bioinformatics and statistical analysis

The sequences were compared with human *DEHAL1* gene sequence (Gene ID: 389434) by DNAMAN software and Chromas software. 100 healthy subjects without thyroid disease were enrolled. SPSS software was applied to determine whether the observed DNA substitutions were mutations or SNPs and whether the difference between them was statistically significant.

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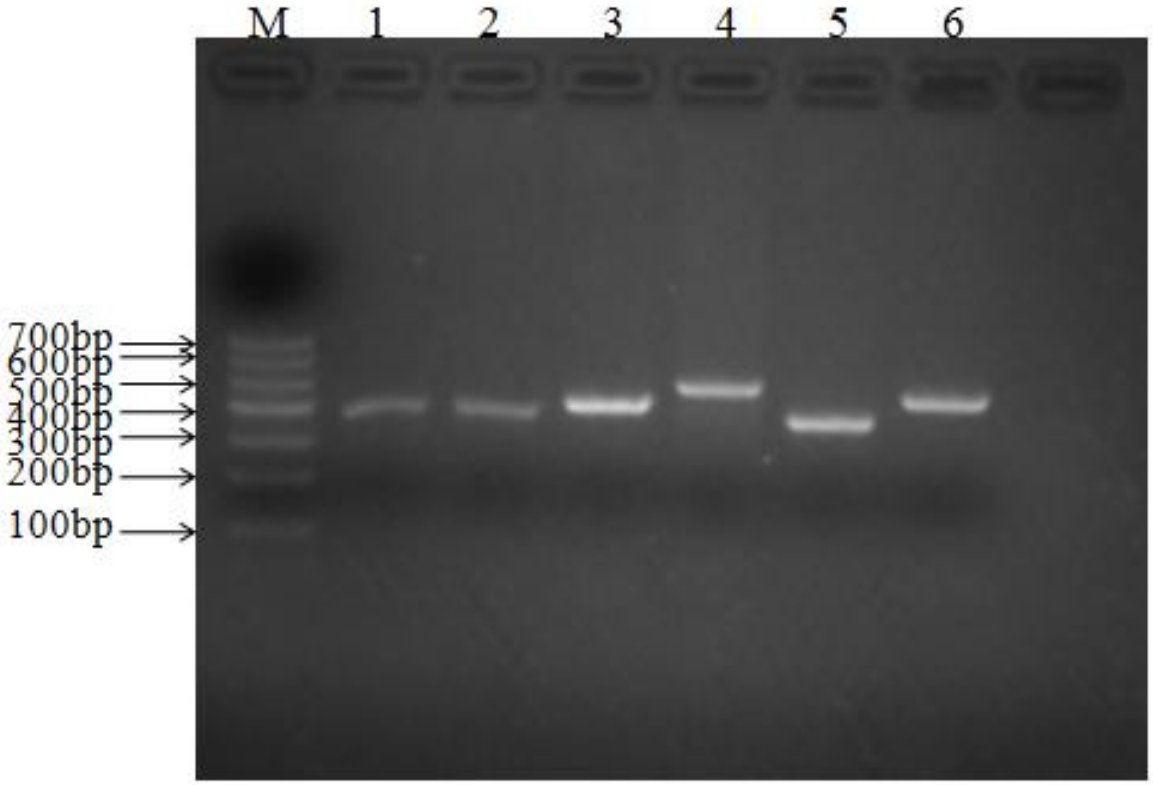
Results

Agarose gel electrophoresis of PCR products

15g/L agarose gel was used to test the PCR products quality, whose band was single and bright can be sequenced (Figure 1).

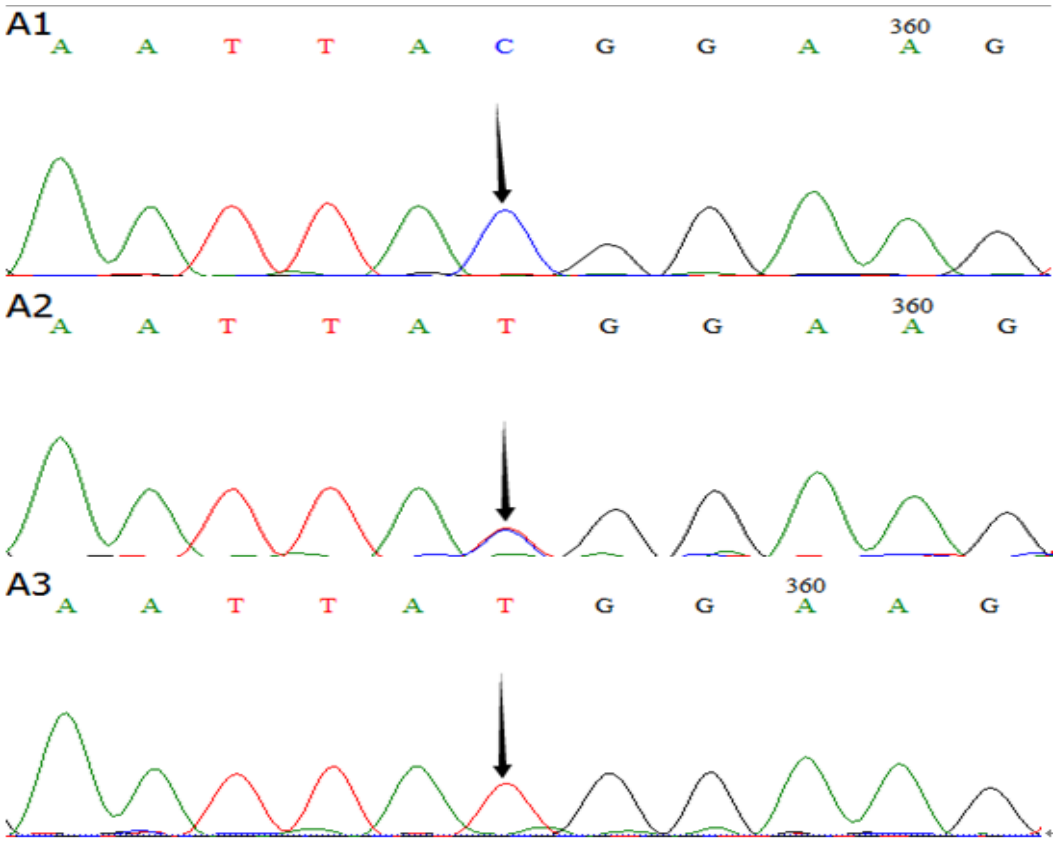


Order from left to right lane is Marker Ⅰ, the amplified products of *DEHAL1* gene exon 1 to 6

Figure 1 Scanning results of agarose gel electrophoresis for *DEHAL1* gene

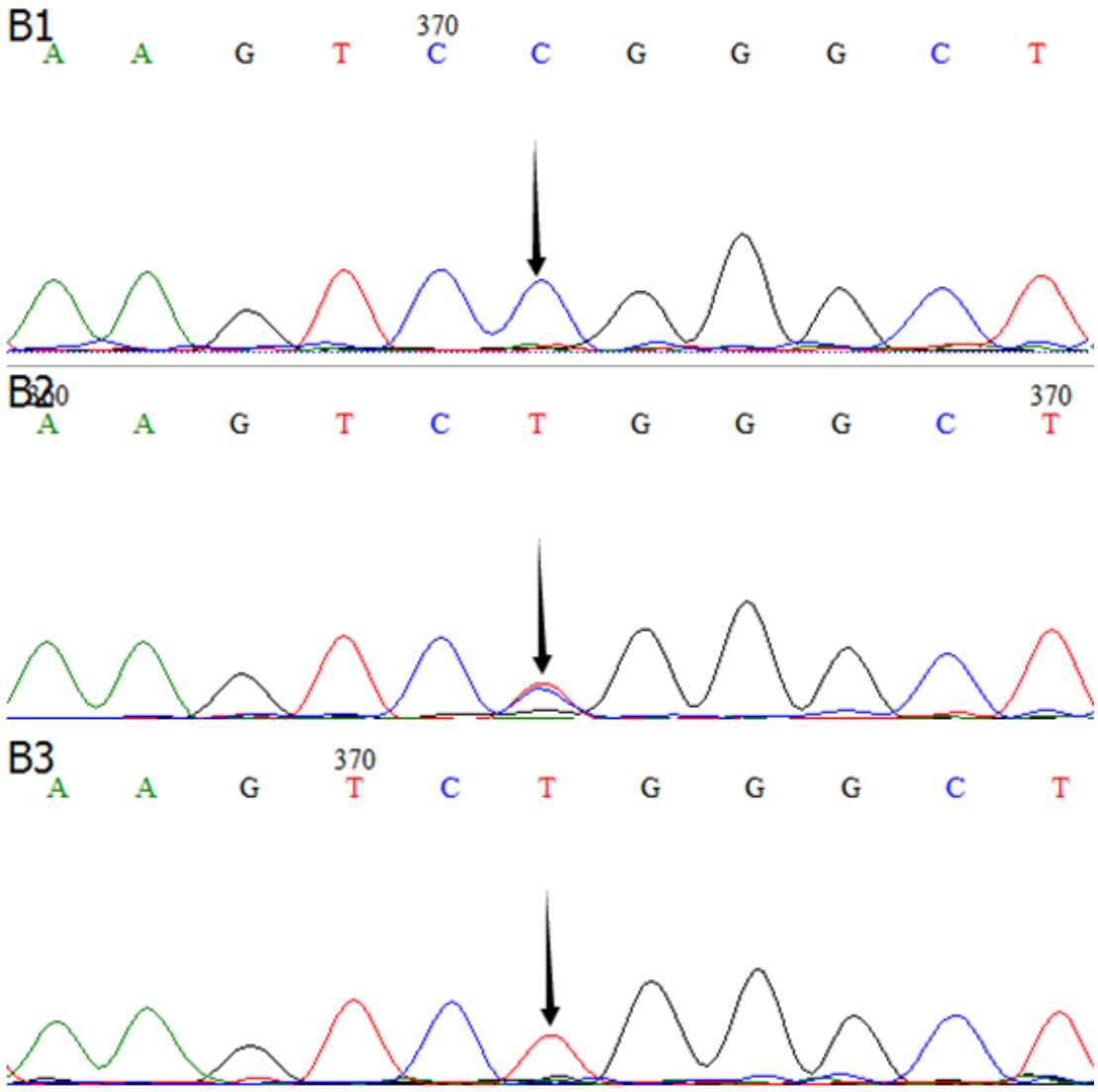
Sequencing results of *DEHAL1*

No *DEHAL1* gene mutation was found in 60 cases of CH with thyroid goiter patients and 100 normal controls, however, two SNPs [rs672766, IVS3+129C>T(as shown in figure 2); rs2076292, IVS3+142C>T (as shown in figure 3)] were found in intron region.



A1: the arrow showed intron genotype IVS3+129C/C A2: the arrow showed intron genotype IVS3+129C/T A3: the arrow showed intron genotype IVS3+129T/T

Figure 2 Sequencing results of SNP (rs2076292, IVS3+142C>T) in *DEHAL1* gene



B1: the arrow showed intron genotype IVS3+142C/C B2: the arrow showed intron genotype IVS3+142C/T B3: the arrow showed intron genotype IVS3+142T/T

Figure 3 Sequencing results of SNP (rs672766, IVS3+129C>A) in *DEHAL1* gene

Bioinformatics and Statistical analysis

Two SNPs (rs672766, IVS3+129C>T; rs2076292, IVS3+142C>T) were found in intron region, this change has no influence on the protein function. The former was found in 7 cases (variation frequency: 11.7%) and 21 normal controls (variation frequency:21%). the latter was found in 4 cases (variation frequency:6.7%) and 17 normal controls (variation frequency:17%). SNP variation frequencies of the CH patients and normal control group were analyzed by chi square test (υ=1), and the result showed that there was no significant difference between the CH patients and normal control group (the former: χ^2=2.26, P > 0.05; the latter: χ^2=3.51, P > 0.05).

Discussion

DEHAL1 gene is located in human chromosome 6q25.1, which contains 6 exons (NM_001164694) and encodes 293 amino acids. In addition to thyroid gland, *DEHAL1* gene has a low level expression in liver, kidney and trachea. The mRNA of DEHAL1 has two subtypes, DEHAL1 and DEHAL1B. Studies have shown that the length difference between the two subtypes is 102 bp and the function of DEHAL1B is inactive[18]. *DEHAL1* gene encodes iodotyrosine deiodinase which is a transmembrane protein with 33kda molecular size and

locates in the apical membrane of the thyroid follicular cells. The transmembrane protein consist of three parts, the extracellular N terminal, a single transmembrane domain and an extracellular C terminal region. The extracellular N terminal is mainly composed of the nitro reductase region.

Thyroid hormone plays a vital role in promoting individual growth, as a result, the lack of thyroid hormone at birth or at an early age will result in cretinism. As we all know, iodine is the essential material involving in thyroid hormone biosynthesis. Two highly specialized systems have been built to ensure the adequate supply of iodine in human body. One is the iodine accumulation system, the iodine derived from digestion and absorption of food will be transported to thyroid by the NIS (sodium/iodine symporter). The other one is the deiodination system, the deiodination of MIT and DIT by iodotyrosine deiodinase can make sure the recovery of iodine, so that the efficiency of the thyroid hormone synthesis can be improved. Once the iodine is absent, the thyroid hormone synthesis will be decreased, the level of serum T4 will decline and TSH will rise, which will lead to CH combined with thyroid goiter.

Patients with ITDD had been found in 1950s[17], Rosenberg[22] had isolated and purified the DEHAL1 from bovine thyroid in 1979. People begin to study the *DEHAL1* gene in molecular level with the development of molecular biology. *DEHAL1* (also known as *IYD*) gene was cloned from human thyroid tissue by the method of SAGE[20] in 2002[18]. In 2008, Moreno JC[21] found three homozygous mutations of *DEHAL1* gene in patients from three unrelated families, including two missense mutations and a frame deletion mutation. Functional studies in vitro showed that the activity of iodotyrosine deiodinase with these three mutations decreased. Afink[17] found a mutation (c.658G>A, p.Ala220Thr) in a consanguineous Moroccan family in 2008. This mutation existed not only in the homozygous but also in a 14 year old boy, which indicated that mutation may have dominant effect. Ainhoa Iglesias[23] thought that heterozygotes stimulated by the external factors such as iodine deficiency or during in adolescence may lead to a certain clinical phenotype. Therefore, it can be hypothesized that phenotypic changes may be caused by environment factors. On the other hand, it may be caused by other gene mutations.

60 cases of CH with thyroid goiter and 100 cases normal control were included in our study to carry out gene mutation screening and no mutation was found, while two SNPs were found in intron region. Existing experimental results suggest that *DEHAL1* gene mutation may not be the main reason lead to CH with thyroid goiter in Shandong province. On one hand , it may be caused by the less sample quantity of the study, therefore, the sample size should be expanded to verify in future research. On the other hand, there may be regional bias, so the study objects should be selected in different regions to eliminate the regional bias. At present, with the development and application of WES (whole genome sequencing technology)[24], which could improve the efficiency of gene sequencing, the diagnosis of some diseases is easier than before. So the WES could be used to detect mutation of some genes related to CH which will provide some convenience for studying the pathogenesis of CH.

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