

## Mutations of Dual Oxidase 2 (DUOX2) Gene among patients with Permanent and Transient Congenital Hypothyroidism

Noushin Rostampour<sup>1</sup>, Mohammad Hassan Tajaddini<sup>2</sup>, Mahin Hashemipour<sup>3</sup>,  
Mansour Salehi<sup>4</sup>, Awat Feizi<sup>5</sup>, Shaghayegh Haghjooy<sup>6</sup>, Roya Kelishadi<sup>7</sup>

### ABSTRACT

**Objective:** The prevalence of congenital hypothyroidism (CH) is high in Isfahan, Iran. In addition, it has different etiologies compared with other countries. The rate of parental consanguinity is also high in the city. Moreover, DUOX2 gene is effective in transient CH and permanent CH due to dysmorphogenesis. Therefore, the aim of this research was to investigate the mutations of DUOX2 gene in patients with transient CH and permanent CH due to dysmorphogenesis.

**Methodology:** In this descriptive, prospective study, patients diagnosed with transient and permanent CH due to dysmorphogenesis during CH screening program were selected. Venous blood samples were obtained to determine the 3 mutations (Q36H, R376W, and D506N) of DUOX2 gene using polymerase chain reaction (PCR) method by specific primers and complementary methods such as restriction fragment length polymorphism (RFLP) and single-strand conformation polymorphism (SSCP).

**Results:** In this study, 25 patients with transient CH and 33 subjects with permanent CH due to dysmorphogenesis were studied. In addition, 30 children were studied as the control group. We did not find any mutations of the 3 mentioned mutations of DUOX2 gene.

**Conclusion:** Considering the findings of the current study, further studies with other methods are required to evaluate other gene mutations such as pendrin, sodium-iodide symporter (NIS) and thyroglobulin.

**KEY WORDS:** Congenital Hypothyroidism, Dual Oxidase 2 (DUOX2) gene, Permanent, Transient.

Pak J Med Sci January - March 2012 (Part-II) Vol. 28 No. 2 287-292

### How to cite this article:

Rostampour N, Tajaddini MH, Hashemipour M, Salehi M, Feizi A, Haghjooy S, Kelishadi R. Mutations of Dual Oxidase 2 (DUOX2) Gene among patients with Permanent and Transient Congenital Hypothyroidism. Pak J Med Sci 2012;28(2):287-292

### INTRODUCTION

Congenital hypothyroidism (CH), with a prevalence of 1/3000-4000 live births, is the most common endocrine disorder and preventable cause of childhood mental retardation.<sup>1,2</sup> CH is considered as a multifactorial disease and many genetical,

environmental and autoimmune factors contribute in its pathogenesis.<sup>3-5</sup> It could be presented in transient or permanent forms. In the transient form, the need for thyroid replacement therapy would be temporary, i.e. for the first 1-3 years, while in the permanent form, treatment would be continued lifelong.<sup>6</sup> The causes of transient CH are excessive or lack of iodine intake and transplacental migration of antibodies and antithyroid drugs.<sup>6</sup> Permanent CH in 85% of cases is associated with a spectrum of thyroid gland developmental defects (dysgenesis) due to abnormal differentiation, migration, or growth of the gland. In 10-20% of cases, it results from defects in one of the multiple steps of thyroid hormone synthesis or defects in the receptor of

#### Correspondence:

Mahin Hashemipour,  
Professor of Pediatric Endocrinology,  
Endocrine and Metabolism Research Center,  
Child Health Promotion Research Center,  
Email: hashemipour@med.mui.ac.ir

- \* Received for Publication: January 18, 2012
- \* Revision Received: February 23, 2012
- \* Revision Accepted: February 27, 2012

thyroid hormone (dyshormonogenesis) which is a heritable disorder represented by goiter in some cases.<sup>6</sup>

Thyroid dysgenesis is a sporadic disorder whose pathogenesis is still unknown. On the contrary, several molecular studies have found different genes, including thyroid stimulating hormone receptor (TSHR), sodium-iodide symporter (NIS), thyroglobulin (TG), thyroid peroxidase (TPO) and pendrin, to be involved in thyroid dysmorphogenesis. Recently, dual oxidase 2 (DUOX2) has been identified as a new gene involved in the etiology of dysmorphogenesis.<sup>7-11</sup>

Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) is considered as an essential compound for thyroid hormone formation and has an important role in the initial step of triiodothyronine (T<sub>3</sub>) and thyroxine (T<sub>4</sub>) synthesis in the follicular lumen of thyrocytes. This biochemical requirement of H<sub>2</sub>O<sub>2</sub> for thyroid hormone production has been known as H<sub>2</sub>O<sub>2</sub>-generating system. DUOX2, a reduced nicotinamide adenine dinucleotide (NAD) phosphate: O<sub>2</sub> oxidoreductase flavoprotein located at the apical plasma membrane of thyrocytes, is a component of the thyroid H<sub>2</sub>O<sub>2</sub>-generating system. It is a 1548-aminoacid polypeptide, including a 26-amino-acid signal peptide. Its gene is located on chromosome 15 and consists of 33 exons encoding an mRNA with 6376 nucleotides. This polypeptide is involved in the Ca<sup>2+</sup>/reduced nicotinamide adenine dinucleotide phosphate-dependent H<sub>2</sub>O<sub>2</sub> generation. H<sub>2</sub>O<sub>2</sub> is used by TPO to catalyze both the iodination of tyrosine residues and the coupling of iodotyrosine residues of TG. DUOX2 is considered as the principal element in generating the H<sub>2</sub>O<sub>2</sub> needed for TPO function.<sup>12-17</sup>

Previous studies have indicated that a defect in the H<sub>2</sub>O<sub>2</sub>-generating system causes congenital hypothyroidism. They have suggested that biallelic mutations of DUOX2 cause permanent congenital hypothyroidism and that monoallelic mutations cause transient congenital hypothyroidism.<sup>18,19</sup> However, some studies have reported transient CH cases, despite biallelic mutations. In addition, familial cases of DUOX2 have also been reported.<sup>18</sup>

The prevalence of CH is high in Isfahan. Moreover, it has different etiologies compared to other countries. The prevalence of transient CH and also the rate of permanent CH due to dysmorphogenesis are high according to CH screening results.<sup>20</sup> On the other hand, high rate of parental consanguinity can be effective on CH incidence.<sup>21</sup> Moreover, DUOX2 gene is involved in transient CH and permanent CH due to dysmorphogenesis.

Therefore, the aim of this research was to investigate the mutations of DUOX2 gene in patients with transient CH and permanent CH due to dysmorphogenesis in Isfahan, Iran.

## METHODOLOGY

In this descriptive, prospective study, patients diagnosed with transient CH or permanent CH due to dysmorphogenesis in Isfahan Endocrine and Metabolism Research Center, during CH screening program (2002-2009) in Isfahan were selected. CH screening program 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 T<sub>4</sub> and TSH levels on their second measurement (TSH > 10 mIU/l and T<sub>4</sub> < 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 T<sub>4</sub> levels were monitored during the follow-up.

Permanent and transient cases of CH were determined at the age of three years old by measuring TSH and T<sub>4</sub> concentrations four weeks after withdrawal of L-T<sub>4</sub> therapy. Patients with elevated TSH levels (TSH > 10 mIU/l) and decreased T<sub>4</sub> levels (T<sub>4</sub> < 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 old after confirming the permanency of CH. Patients with thyroid gland of normal size were considered to have dysmorphogenesis.<sup>20</sup>

The protocol was approved by the Institutional Review Board and Medical Ethics Committee of Isfahan University of Medical Sciences. Written consents were obtained from the parents of CH patients.

The selected CH patients were examined by a pediatrician and the demographic characteristics and screening findings regarding the level of TSH and T<sub>4</sub>, parental consanguinity and the etiology of CH were recorded using a questionnaire.

Venous blood samples were obtained from the selected patients. The samples were transferred to the Department of Genetics, Isfahan University of Medical Sciences for molecular analysis and determining the 3 mutations (Q36H, R376W, and D506N) of DUOX2 gene using the polymerase chain reaction (PCR) method by specific primers and complementary methods such as restriction fragment length

Table-I: Demographic characteristics of patients with transient and permanent CH due to dyshormonogenesis and the control group.

	Patients with transient CH	Patient with permanent CH due to dyshormonogenesis	Control group
Age (months)	68.2 ± 25.5	64.6 ± 23.7	69.3 ± 27.2
Sex (male/female)	18/7	15/18	19/11
Parental consanguinity (%)	69%	62%	39%
TSH (mIU/l)			
-primary	25.5 ± 19.7	47.3 ± 46.1	4.5 ± 2.7
-after treatment interruption	3.9 ± 2.0	36.5 ± 30.7	-
T4 (µg/dl)			
-primary	5.7 ± 3.5	6.2 ± 3.5	11.6 ± 3.9
-after treatment interruption	8.1 ± 1.9	7.1 ± 2.9	-

polymorphism (RFLP) and single-strand conformation polymorphism (SSCP).

**Genetic study:** DNA was extracted from peripheral blood by the QIAamp DNA Blood Mini Kit (Qiagen, Germany). Real-time PCR and melting curve analysis were performed in the Corbett Rotor-Gene 6000 instrument (Corbett Research, Sydney, Australia).

Primers were designed by Beacon Designer 7.91 to flank the coding regions (Premier Biosoft International, USA) and synthesized by TIB MolBiol (Germany). The primers included Q36H forward primer (5'-GGGAGGGGTAGCTGGGAGC-3') and reverse primer (5'- CCGCTCAGGGC-CTTCGC-3'), R376W forward primer (5'-TCC-CTCACCACATCCTTTGTTCTCA-3') and reverse primer (5'- TGTGCTTTTCCCAGCCIGTGTG-3'), and D506N forward primer (5'-CATGGGGAC-CCTGGACCC) and reverse primer (5'- GTGTG-GTGGGCTGACTGGG-3'). The final optimal reaction conditions were empirically determined. The reaction mixture used was Type-it HRM Kit (Qiagen, Germany).

The amplification mixture of a total volume of 25 µL included 12.5 µL of high resolution melt (HRM) PCR master mix, 1.75 µL of 10 µM primer mix, 2 µL of genomic DNA as template and 8.25 µL of RNase-free water. The PCR cycling conditions are reported in Tables II-IV. The HRM analysis was performed

by instrument software, which allows clustering the samples into groups on the basis of difference plots obtained by analyzing the differences in melting curve shapes.

One sample from each group with less than 90% confidence was confirmed by sequencing PCR products in the 3730xl Genetic Analyzer (Bioneer, South Korea).

**Statistical Analysis:** The obtained data was analyzed using the SPSS<sub>13</sub> (SPSS Inc., Chicago, IL, U.S.A.).

## RESULTS

In this study, 25 patients with transient CH and 33 cases of permanent CH due to dyshormonogenesis were studied. Thirty children were studied as the control group. Demographic characteristics of the studied population are presented in Table-I. The difference plot showed the approximately same shapes detected in the same amplicons (Fig. 1-3). Sequencing analysis did not show different results. We did not find any of the three mentioned DUOX2 gene mutations (Q36H, R376W, and D506N).

## DISCUSSION

Similar to other etiological investigations during the CH screening in Isfahan, this study evaluated the role of three mutations of DUOX2 (Q36H, R376W, and D506N) in transient CH patients and permanent

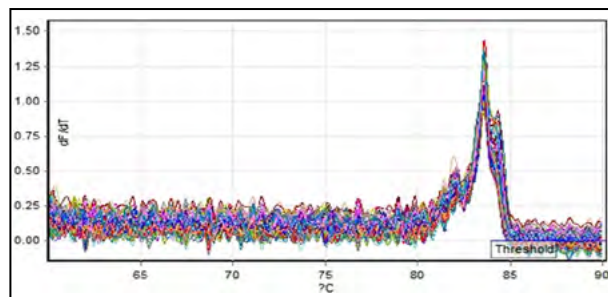


Fig.1: Sequencing analysis of the Q36h mutation.

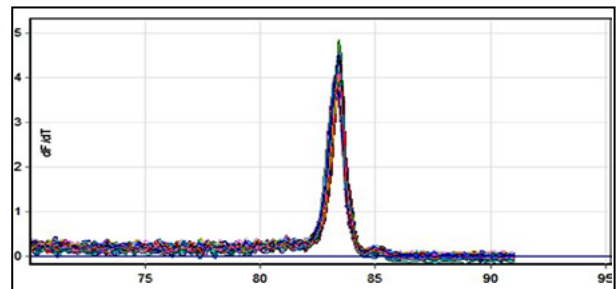


Fig.2: Sequencing analysis of the R376W mutation.

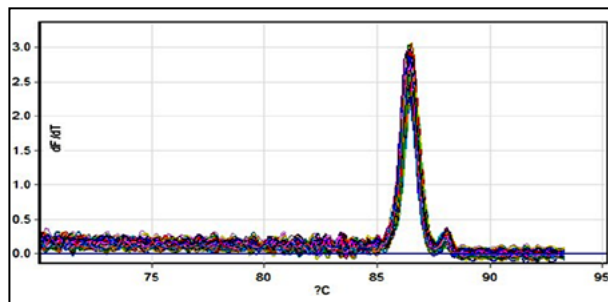


Fig.3: Sequencing analysis of the D506N mutation. CH patients due to dyshormonogenesis. According to our results, the studied population, including both transient and permanent CH patients, did not have any of the mentioned mutations.

Many human and animal studies have suggested the important role of DUOX2 gene in the synthesis of thyroid hormone and the etiology of CH.<sup>22</sup> The mentioned association have mostly been recognized by the neonatal screening test for CH since it is difficult to diagnose adult cases of DUOX2 mutation. The role of DUOX2 gene mutation in CH was reported by Moreno et al. in 2002 for the first time.<sup>18</sup> Since then, several studies indicated different mutations of DUOX2 gene to be responsible for transient and permanent CH. Overall, 20 mutations have been reported from which 11 are located in the first long intracellular loop, 8 in the extracellular peroxidase-like domain and one in the second intracellular loop. The types of identified mutations include missense, nonsense, frame shift and splice-site mutations.<sup>23</sup>

It has been proposed that biallelic and monoallelic mutations of the gene cause permanent and transient CH, respectively. However, some studies have also reported biallelic mutations of DUOX2 gene among transient CH cases, which suggests the presence of an alternative mechanism for producing H<sub>2</sub>O<sub>2</sub> in complete defect of DUOX2 gene. Familial cases of CH with heterozygous mutations of DUOX2 have also been reported.<sup>18,19</sup>

In addition, an identical DUOX2 mutation could be present with a high intra- and inter-familial phenotypic variability. Vigone et al have reported that

Table-III: The PCR cycling conditions for determining the R376W mutation.

Cycle	Cycle Point
Hold @ 95°C, 5 min 0 secs	
Cycling (45 repeats)	Step 1 @ 95°C, hold 11 secs Step 2 @ 59°C, hold 24 secs Step 3 @ 72°C, hold 15 secs,
Melt (70-95°C), hold secs on the 1st step, hold 2 secs on next steps, Melt A([HRM])	

Table-II: The PCR cycling conditions for determining the Q36H mutation.

Cycle	Cycle Point
Hold @ 95°C, 6 min 0 secs	
Cycling (45 repeats)	Step 1 @ 95°C, hold 12 secs Step 2 @ 55°C, hold 31 secs Step 3 @ 72°C, hold 16 secs,
Melt (60-90°C), hold secs on the 1st step, hold 2 secs on next steps, Melt A([HRM])	

two siblings with the same identical DUOX2 mutation had different clinical phenotypes.<sup>24</sup> This variability of the DUOX2 phenotype could be explained by some hypotheses such as the existence of other H<sub>2</sub>O<sub>2</sub> generating systems, the different requirements for thyroid hormones synthesis according to age, the ethnicity and the intake of iodine.<sup>24,25</sup> Genetic studies have showed some factors as DUOX2 maturation factors which enhance the endoplasmic reticulum (ER)-to-Golgi transition, maturation and surface expression of functional DUOX2 in a heterologous cell system. In this regard, three natural missense mutations including Q36H, R376W, and D506N have been reported. They were also evaluated in the current study. These mutations cause partial (D506N) or complete (Q36H and R376W) loss of H<sub>2</sub>O<sub>2</sub> generating system activity which consequently results in iodine organification defect. The defects caused by these mutations are made by trafficking defects of the mutant proteins resulting in complete retention in the ER due to Q36H and R376W mutations or reduced plasma membrane translocation due to D506N mutation.<sup>26</sup>

Considering the key role of the mentioned mutations in the synthesis of thyroid hormone and iodine organification process, we investigated the role of these three mutations of the peroxidase-like domain. The mentioned three mutations have been identified during the CH screening programs. For the first time, the mutation of R376W, Q36H and D506N were reported by Vigone et al in 2005,<sup>24</sup> Varela et al in 2006,<sup>27</sup> and Pfarr et al in 2006,<sup>28</sup> respectively. Afterwards, Grasberger et al performed a functional study and analyzed the three mutations in Cos-7

Table-IV: The PCR cycling conditions for determining the D506N mutation.

Cycle	Cycle Point
Hold @ 95°C, 5 min 0 secs	
Cycling (45 repeats)	Step 1 @ 95°C, hold 11 secs Step 2 @ 59°C, hold 24 secs Step 3 @ 72°C, hold 15 secs,
Melt (70-95°C), hold secs on the 1st step, hold 2 secs on next steps, Melt A([HRM])	

cells. According to their findings, due to complete inhibition of the transition of DUOX from the ER to the plasma membrane by Q36H and R376W mutations or partial (D506N) inhibition of H<sub>2</sub>O<sub>2</sub>-generating system activity by these mutations, these DUOX2 mutants were retained within the ER. They suggested that post-translational processing of the peroxidase-like domain had an important role in the exit of DUOX2 from ER.<sup>26</sup>

We did not find any mutation of the mentioned three mutations (Q36H, R376W, and D506N) of DUOX2 gene among either group of transient or permanent CH patients in the current study. The most important explanation for this finding could be the ethnical variation in the genes involved in thyroid hormonogenesis as reported by other studies.<sup>29</sup> Mutations in other genes involved in the pathogenesis of transient and permanent CH due to dys hormonogenesis, such as NIS gene, pendrin gene (PDS) and thyroglobulin gene, may have had a role in our studied population. However, such hypothesis needs further investigations.

On the other hand, previous studies have indicated that some factors such as age, ethnicity, and iodine intake could explain the different phenotype variations of an identical DUOX2 gene mutation. The mentioned factors could also have a role in different mutations of thyroid hormone synthesis. The sample size was enough for the purpose of the study and so it could not be considered as a limiting factor in this field.

Our finding could also be explained by different factors related to the method of the genetic study. In the current study, only the exonic parts of the genes were analyzed. However, mutations in intronic sequences or in the promoter region and unexamined regulatory regions of the three studied genes could have been the causes of thyroid dys hormonogenesis or transient CH in these patients.<sup>30</sup>

Another explanation is that although the method used in this study has a high sensitivity, it is highly dependent on laboratory conditions. Therefore, some mutations with slight impacts on changes in single-strand confirmation could not be detected by this method. Thus, some existing mutations might have remained unidentified with SSCP.<sup>31</sup>

The limitation of this research was that we did not determine iodine organification defects in the studied patients due to insufficient facilities to perform perchlorate discharge test. In addition, it is necessary to examine other identified mutations of DUOX2 gene in the studied population.

In conclusion, the results of this study

indicated that the mutations of the peroxidase-like domain of DUOX2 did not have a role in the pathogenesis of transient and permanent CH due to dys hormonogenesis in this population. However, for more conclusive results, further studies using another screening method besides SSCP, and screening of intronic mutations and other identified DUOX2 gene mutations on the other sites of the gene are recommended. Investigation of other gene mutations responsible for thyroid dys hormonogenesis and transient CH such as pendrin, NIS and thyroglobulin is recommended, too.

#### ACKNOWLEDGEMENT

The authors would like to thanks Dr. Hossein Saneian, Dr. Silva Hovsepian and Prof. Massoud Amini for their valuable help and assistance in conducting this study.

#### REFERENCES

1. Knobel M, Medeiros-Neto G. An outline of inherited disorders of the thyroid hormone generating system. *Thyroid* 2003;13(8):771-801.
2. Castanet M, Polak M, Bonaïti-Pellié C, Lyonnet S, Czernichow P, Léger J. Nineteen years of national screening for congenital hypothyroidism: familial cases with thyroid dysgenesis suggest the involvement of genetic factors. *J Clin Endocrinol Metab* 2001;86(5):2009-2014.
3. Benvenega S, Ordookhani A, Pearce EN, Tonacchera M, Azizi F, Braverman LE. Detection of circulating autoantibodies against thyroid hormones in an infant with permanent congenital hypothyroidism and her twin with transient congenital hypothyroidism: possible contribution of thyroid hormone autoantibodies to neonatal and infant hypothyroidism. *J Pediatr Endocrinol Metab* 2008;21(10):1011-1020.
4. Carranza D, Van Vliet G, Polak M. Congenital hypothyroidism. *Ann Endocrinol (Paris)* 2006;67(4):295-302.
5. Kopp P. Perspective: genetic defects in the etiology of congenital hypothyroidism. *Endocrinology* 2002;143(6):2019-2024.
6. Park SM, Chatterjee VKK. Genetics of congenital hypothyroidism. *J Med Genetics* 2005;42:379-389.
7. Abramowicz MJ, Targovnik HM, Varela V, Cochaux P, Krawiec L, Pisarev MA, et al. Identification of a mutation in the coding sequence of the human thyroid peroxidase gene causing congenital goiter. *J Clin Invest* 1992;90(4):1200-1204.
8. Ieiri T, Cochaux P, Targovnik HM, Suzuki M, Shimoda S, Perret J, et al. A 3 splice site mutation in the thyroglobulin gene responsible for congenital goiter with hypothyroidism. *J Clin Invest* 1991;88(6):1901-1905.
9. 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(2):124-125.
10. Everett LA, Glaser B, Beck JC, Idol JR, Buchs A, Heyman M, et al. Pendred syndrome is caused by mutations in a putative sulphate transporter gene (PDS). *Nat Genet* 1997;17(4):411-422.

11. Nose O, Harada T, Miyai K, Hata N, Ogawa M, Maki I, et al. Transient neonatal hypothyroidism probably related to immaturity of thyroidal iodine organification. *J Pediatr* 1986;108(4):573-576.
12. Corvilain B, van Sande J, Laurent E, Dumont JE. The H<sub>2</sub>O<sub>2</sub>-generating system modulates protein iodination and the activity of the pentose phosphate pathway in dog thyroid. *Endocrinology* 1991;128(2):779-785.
13. Dupuy C, Ohayon R, Valent A, Noël-Hudson MS, Deme D, Virion Q. Purification of a novel flavoprotein involved in the thyroid NADPH oxidase. *J Biol Chem* 1999;274(52):37265-37269.
14. De Deken X, Wang D, Many MC, Costagliola S, Libert F, Vassart G, et al. Cloning of two humanthyroidcDNA encoding new members of the NADPH oxidase family. *J Biol Chem* 2000;275(30):23227-23233.
15. Kusakabe T. Deficient cytochrome b5 reductase activity in nontoxic goiter with iodide organification defect. *Metabolism* 1975;24(10):1103-1113.
16. Niepomnyszcz H, Targovnik HM, Gluzman BE, Curutchet P. Abnormal H<sub>2</sub>O<sub>2</sub> supply in the thyroid of a patient with goiter and iodine organification defect. *J Clin Endocrinol Metab* 1987;65(2):344-348.
17. Figueiredo MD, Cardoso LC, Ferreira AC, Campos DV, da Cruz Domingos M, Corbo R, et al. Goiter and hypothyroidism in two siblings due to impaired Ca(+2)/NAD(P)H-dependent H(2)O(2)-generating activity. *J Clin Endocrinol Metab* 2001;86(10):4843-4848.
18. Moreno JC, Bikker H, Kempers MJ, van Trotsenburg AS, Baas F, de Vijlder JJ, et al. Inactivating mutations in the gene for thyroid oxidase 2 (THOX2) and congenital hypothyroidism. *N Engl J Med* 2002;347(2):95-102.
19. Maruo Y, Takahashi H, Soeda I, Nishikura N, Matsui K, Ota Y, et al. Transient Congenital Hypothyroidism Caused by Biallelic Mutations of the Dual Oxidase 2 Gene in Japanese Patients Detected by a Neonatal Screening Program. *Clin Endocrinol Metab* 2008;93(11):4261-4267.
20. Hashemipour M, Hovsepian S, Kelishadi R, Iranpour R, Hadian R, Haghghi S, et al. Permanent and transient congenital hypothyroidism in Isfahan-Iran. *J Med Screen* 2009;16(1):11-16.
21. Hashemipour M, Amini M, Talaie M, Kelishadi R, Hovespian S, Iranpour R, et al. Parental consanguinity among parents of neonates with congenital hypothyroidism in Isfahan. *East Mediterr Health J* 2007;13(3):567-574.
22. Johnson KR, Marden CC, Ward-Bailey P, Gagnon LH, Bronson RT, Donahue LR. Congenital hypothyroidism, dwarfism, and hearing impairment caused by a missense mutation in the mouse dual oxidase 2 gene, Duox2. *Mol Endocrinol* 2007;21(7):1593-1602.
23. Ohye H, Sugawara M. Dual oxidase, hydrogen peroxide and thyroid diseases. *Exp Biol Med (Maywood)* 2010;235(4):424-433.
24. Vigone MC, Fugazzola L, Zamproni I, Passoni A, Di Candia S, Chiumello G, et al. Persistent mild hypothyroidism associated with novel sequence variants of the DUOX2 gene in two siblings. *Hum Mutat* 2005;26(5):395.
25. Fugazzola L, Muzza M, Weber G, Beck-Peccoz P, Persani L. DUOX2 defects: Genotype-phenotype correlations. *Ann Endocrinol (Paris)* 2011;72(2):82-86.
26. Grasberger H, De Deken X, Miot F, Pohlenz J, Refetoff S. Missense mutations of dual oxidase 2 (DUOX2) implicated in congenital hypothyroidism have impaired trafficking in cells reconstituted with DUOX2 maturation factor. *Mol Endocrinol* 2007;21(6):1408-1421.
27. Varela V, Rivolta CM, Esperante SA, Gruñeiro-Papendieck L, Chiesa A, Targovnik HM. Three mutations (p.Q36H, p.G418fsX482, and g.IVS19-2A>C) in the dual oxidase 2 gene responsible for congenital goiter and iodide organification defect. *Clin Chem* 2006;52(2):182-191.
28. Pfarr N, Korsch E, Kaspers S, Herbst A, Stach A, Zimmer C, et al. Congenital hypothyroidism caused by new mutations in the thyroid oxidase 2 (THOX2) gene. *Clin Endocrinol (Oxf)* 2006;65(6):810-815.
29. Tonacchera M, De Marco G, Agretti P, Montanelli L, Di Cosmo C, Freitas Ferreira AC, et al. Identification and functional studies of two new dual-oxidase 2 (DUOX2) mutations in a child with congenital hypothyroidism and a eutopic normal-size thyroid gland. *J Clin Endocrinol Metab* 2009;94(11):4309-4314.
30. Niu DM, Hwang B, Chu YK, Liao CJ, Wang PL, Lin CY. High Prevalence of a Novel Mutation (2268 insT) of the Thyroid Peroxidase Gene in Taiwanese Patients with Total Iodide Organification Defect, and Evidence for a Founder Effect. *J Clin Endocrinol Metab* 2002;87(9):4208-4212.
31. Nollau P, Wagener C. Methods for detection of point mutations: performance and quality assessment. IFCC Scientific Division, Committee on Molecular Biology Techniques. *Clin Chem* 1997;43(7):1114-1128.

#### Author's Contributions:

Noushin Rostampour, Mahin Hashemipour, Mansour Salehi, Roya Kelishadi; Concepts, design, literature search, clinical and experimental studies, data collection and analysis, manuscript preparation, editing and review  
 Mohammad Hassan Tajaddini, Shaghayegh Haghjooy; Design, literature search, experimental and laboratory studies, data collection, manuscript preparation, editing and review.  
 Awat Feizi; Design, literature search, data analysis, statistical analysis, manuscript preparation, editing and review.

---

#### Authors:

1. Noushin Rostampour, Pediatrician, Department of Pediatrics, School of Medicine,
2. Mohammad Hassan Tajaddini, MSc Student in Clinical Biochemistry, School of Pharmacy and Isfahan Pharmaceutical Sciences Research Center,
3. Mahin Hashemipour, MD, Professor of Pediatric Endocrinology, Endocrine and Metabolism Research Center, Child Health Promotion Research Center,
4. Mansour Salehi, PhD, Associate Professor of Molecular Genetics, Department of Genetics and Molecular Biology, School of Medicine,
5. Awat Feizi, PhD, Assistant Professor of Biostatistics, Department of Epidemiology and Biostatistics, School of Public Health,
6. Shaghayegh Haghjooy, MD, PhD, Applied Physiology Research Center and Dept. of Physiology, School of Medicine,
7. Roya Kelishadi, MD, Professor of Pediatrics, Department of Pediatrics, Child Health Promotion Research Center, Isfahan University of Medical Sciences, Isfahan, Iran.