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Prevalence of THRB Gene Mutations in Misan Province Thyroidism Patients

Duha T. Al-Taie[1] , Zahr'aa Kateb Jumaa[2] , Rehal Jehad Husain[3],Eman H. Rahi[4]

[1,3] Ministry of Education, Open Educational College, Iraq

[1,2,3] Imam Ja'afar Al-Sadiq University, College of Health and Medical Technologies

[4] Department of Basic Sciences, College of Nursing, University of Basrah, Basrah, Iraq.

Email: duhaaltaie6@gmail.com

Abstract. General Background: Thyroid disorders such as hypothyroidism and hyperthyroidism are common endocrine abnormalities characterized by disrupted levels of triiodothyronine (T3), thyroxine (T4), and thyroid-stimulating hormone (TSH). Specific Background: Mutations in the thyroid hormone receptor beta (THRB) gene are implicated in thyroid hormone resistance syndromes and potentially in other thyroid dysfunctions. Knowledge Gap: However, there is limited data regarding the prevalence of THRB gene mutations among thyroid disorder patients in Misan Province, Iraq. Aims: This study aimed to evaluate the presence of THRB gene mutations among women diagnosed with thyroid disorders and assess corresponding hormonal variations across age groups. Results: Hormonal analysis revealed significant deviations in FT3, FT4, and TSH levels in both hypothyroid and hyperthyroid patients across multiple age groups. Molecular analysis, however, did not detect any pathogenic mutations in the THRB gene. Novelty: Despite the identification of several unique haplotypes among patients, none corresponded to known pathogenic mutations, suggesting possible population-specific genetic profiles without direct impact on receptor function. Implications: These findings underscore the need for broader genetic screening beyond THRB in diagnosing thyroid disorders and highlight the limited role of THRB mutations in the pathogenesis of thyroid dysfunction in this regional cohort.

Highlights:

1. No mutations were found in the THRB gene in patients with hypothyroidism and hyperthyroidism.
2. Hormone analysis showed significant differences in FT3, FT4, and TSH levels between age groups.
3. There were ten varying THRB gene haplotypes, but no mutations were detected.

Keywords: Thyroid disorders, Thyroid hormone, TRHB gene, Novel mutation, Hyperthyroidism

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Introduction

The thyroid gland, which is the largest and important endocrine gland in the human body (typically 15–20 g), is located in the anterior side of the neck, right below the larynx. It has two lobes connected by an isthmus, which has the shape of a butterfly [1].

The thyroid gland synthesizes and secretes two major hormones, known as triiodothyronine (T3) and thyroxine, which can sometimes be referred to as tetraiodothyronine (T4) [2, 3]. T3 and T4 are the two most important hormones produced by the thyroid gland, which play an essential role in regulating the metabolic function, development, and growth [4]. On the other hand, thyroid dysfunction is caused by an imbalance in the thyroid gland activity, hypothyroidism and hyperthyroidism, leading to an increase or a decrease in the thyroid hormones secretion [5, 6]. The thyroid gland also secretes calcitonin, an important hormone for calcium metabolism [7].

Hypothyroidism is a common endocrine disorder resulting from a primary process when the thyroid gland fails to generate enough thyroid hormones (T3\T4) [8]. In contrast, when of thyroid gland produces more hormones than the body requirement leads to a condition of hyperthyroidism [9].

There are several of the mutations associated with thyroid disease have been diagnosed, most of these mutations occur in the genes responsible for synthesis thyroid hormones (T3\T4) and in the TSH receptor, among these genes are the thyroglobulin (*TG*) gene and thyroid hormone receptor (*TSHR*) gene [10, 11], and_thyroid hormone receptor beta (*THRB*) gene [12, 13].

Materials and methods:

Study Samples

The subjects of this study involved 150 women, these women divided in to control group (No . 50) and patients groups (No. 100) (50 hypothyroidism and 50 hyperthyroidism), at the ages ranged between (15-64) years, the ages were divided into five groups, also each group was divided into five age categories as follows: first group (15-24) year, second group (25-34) year, third group (35-44) year, fourth group(45-54) and fifth group (55-64) year and each category consist of (12), (39), (39), (42) and (18) women, respectively.

Women with thyroid disorders have been checked medically by specialized doctors and have been diagnosed with hypothyroidism and hyperthyroidism. On the other hand, some women were excluded because they suffer from diabetes and hypertension. A questionnaire was designed to obtain the actual information of women in the control and patient groups.

Blood Samples Collection

About 5 milliliters of whole blood were obtained from a medical syringe of each subject (patients and control group women). Five ml of venous blood was divided into two volumes. Three mL of blood sample was put into a gel tube for 20 minutes at room temperature for clotting. Then, transported into a centrifuge, which was fixed at 3000 rpm for 10 minutes to collect the serum. In addition, two mL of whole blood were put into an EDTA vial, kept in - 20 ˚C for a longer period to use it in a subsequent molecular study.

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Physiological study:-

Concentration of thyroid hormones parameters measured by the chemiluminescent automated immunoassay system (Cobas e 411).

Molecular study: -

DNA Extraction

DNA was extracted from all samples using a DNA extraction kit supplied by Geneaid.

Polymerase Chain Reaction process

The Polymerase Chain Reaction (PCR) has been done using primers for the *THRB* gene as shown in Table 1.

Table (1) The primer sequence used in PCR work

Primers		Sequences	Length (bP)	References
THRB	F	FGGTTGTCGAAAGTCTGCAG	20	(Yang, and Yan, 2016)
	R	RGTATCCACCAAAGGCGG	18	

The amplification was performed in volume of 50 µl of reaction mixtures.

After completing the additions, the components were mixed using the Vortex Thermolyne to mix the components inside the tube. After that, it was placed in the Thermo cycler and set the apparatus working on the program as shown in the table (2).

Table (2) Programs of PCR work

Gene amplification	Steps	Temperature	Time	No. Cycle
THRB	Initial denaturation	95 °C	4 min	1
	Denaturation	95 °C	40 sec.	35
	Annealing	60°C	40 sec.	
	Extension	72°C	1 min	
	Extension	72°C	10 min	1

Agarose Gel Electrophoresis

After genomic DNA extraction, agarose gel electrophoresis was applied to confirm the presence of the extracted DNA according to Sambrook *et al.* (1989).

Analysis of nucleotide sequence

The samples (20 µl (were sent to the Macrogen company (South Korea) to perform the

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sequencing analysis.

Statistical analysis:

Statistical analysis in the present study was performed by using one-way ANOVA by general linear model procedure using Statistical Package for Social Sciences (SPSS) version 31. The comparisons between means scores were made using revised least significant differences (RLSD). The difference was considered to be significant at P≤ 0.05 using SPSS.

Mutations and their types were analyzed using Mutation Surveyor Software V.5.2. As for Genetic diversity to account of haplotypes diversity (HD) and nucleotide diversity was done by using a program DnaSP V.5.10, while, the haplotypes network is drawn using a program network V.5.0.0., also, PyMol was used to plot the shape of a protein.

Results

Thyroid hormones concentration

The results of thyroid hormonal analysis showed in figure (1) table (3) a significant decrease (P≤0.05) in the level of Free Triiodothyronine (FT3) in the ages groups (15-24), (35-44) and (45-54) yrs, and did not differ significantly in the ages groups (25-34) and (55-64) yrs with hypothyroidism patients as compared with the control group. On the other hand, hyperthyroidism patients, as compared with the control group, showed a significant difference (P≤0.05) in all the age groups except the age group (15-24) yrs did not show a significant difference.

Figure (2), Table (3) show the values of FT4 concentration decreased significantly (P≤0.05) in all the ages groups except for the age group (25-34) yrs did not significantly differ in hypothyroidism patients as compared with the control group; whereas, a significant difference (P≤0.05) increased in all the ages groups (15- 64) yrs with hyperthyroidism patients as compared with the control group.

Additionally, the TSH concentration significantly increases in hypothyroidism and significantly decreases in hyperthyroidism in all the age groups 15-64 years as compared with the control group (Figure 3, Table 3).

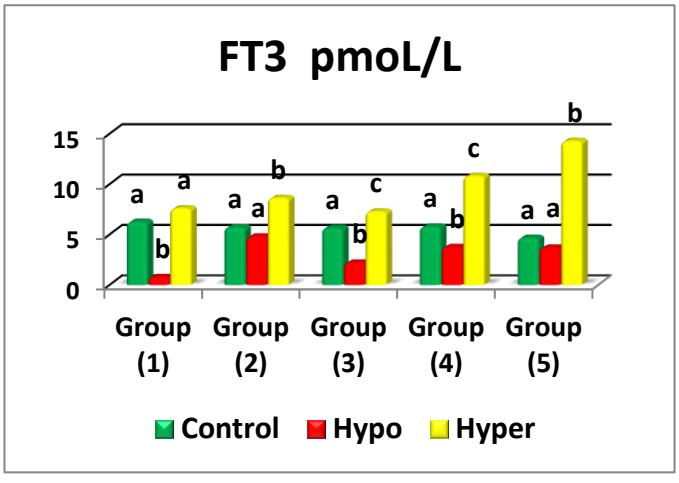

Figure (1) The FT3 concentration in control, hypo, and hyperthyroidism women.

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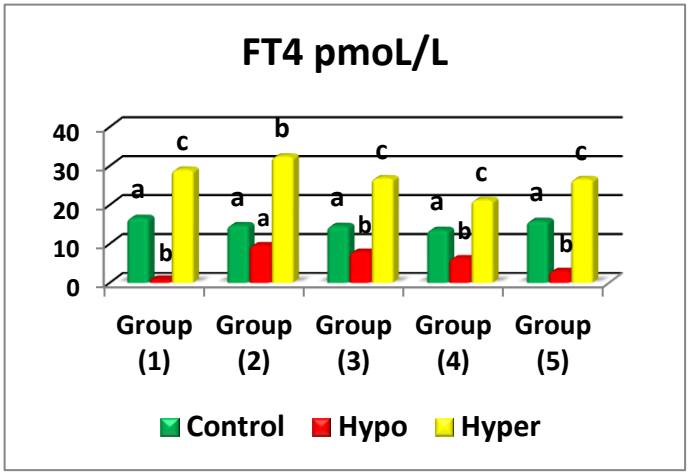

Figure (2) The FT4 concentration in control, hypo, and hyperthyroidism women.

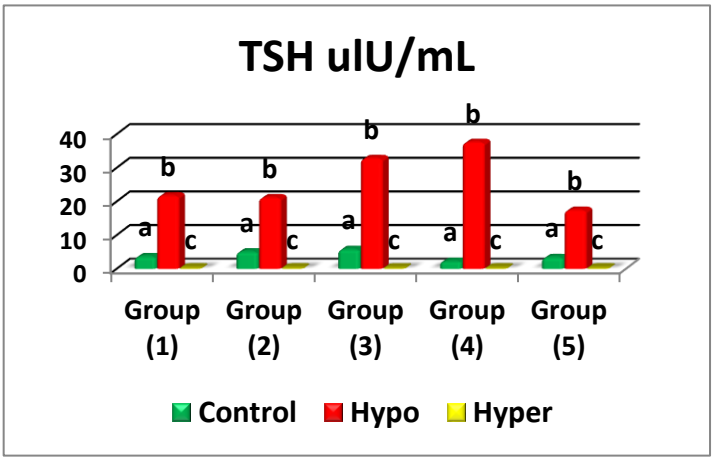

Figure (3) The TSH concentration in control, hypo, and hyperthyroidism women.

Table (3): The values of serum thyroid hormone levels among hypothyroidism, hyperthyroidism, and control (Mean ± SD)

Groups \ Parameters		FT3 pmoL/L	FT4 pmoL/L	TSH ulU/mL
Group (1) (15-24) yrs N= 12	Control	6.26 ± a 0.122	16.64 ± a 0.32	3.67± a 0.27
	Hypo	0.76 ± b 0.40	0.82 ± b 0.27	21.73± b 0.76
	Hyper	7.58 ± a 1.02	29.01± c 0.48	0.004 ± c 0.0009
	R.L.S.D	1.72	0.99	1.26
Group	Control	5.73 ±a 0.22	14.76 ± a 0.69	5.09± a 0.16
	Hypo	4.80 ±a	9.63 ± a	21.16± b

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(2) (25-34) yrs N= 39		0.29	1.08	0.46
	Hyper	8.62 ± b 1.04	32.40± b 4.87	0.003± c 0.0004
	R.L.S.D	1.69	7.40	0.69
Group (3) (35-44) yrs N= 39	**Control**	5.66± a 0.16	14.57± a 0.64	5.80± a 0.35
	Hypo	2.21± b 0.52	7.94± b 0.80	32.83± b 2.63
	Hyper	7.28± c 0.50	26.82± c 1.86	0.002± c 0.0003
	R.L.S.D	1.06	3.05	3.81
Group (4) (45-54) yrs N = 42	**Control**	5.79± a 0.18	13.56± a 0.49	2.24± a 0.42
	Hypo	3.76± b 0.17	6.27± b 1.23	37.62± b 8.14
	Hyper	10.81± c 0.52	21.23± c 1.80	0.004± c 0.0006
	R.L.S.D	0.83	3.19	3.24
Group (5) (55-64) yrs N= 18	**Control**	4.67± a 045	15.86 ± a 0.60	3.39± a 0.47
	Hypo	3.70± a 0.20	3.01± b 1.10	17.44± b 1.20
	Hyper	14.28± b 1.28	26.59± c 4.99	0.01± c 0.007
	R.L.S.D	2.05	8.59	1.92

- Different letters refer to significant differences among groups at the level of (P≤0.05)

4.3.3. *THRB* gene and Thyroid disorder

4.3.3.1. *THRB* gene

The complete sequence *THRB* gene and mRNA–THRB sequences, from the base (1-015832.1), for a human were obtained from NCBI (www.ncbi.com).

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4.3.3.2. Changes in the *THRB* gene

The exon 10 region sequence was analyzed for the *THRB* gene by a thermal cycler apparatus, and then the results were compared for 22 samples (10 hypothyroidism, 10 hyperthyroidisms, 2 control) directly with reference to the gene THRB by the program of the mutation survey software. Figure (4) shows the electrophoresis of the PCR amplification product of the *THRB* gene.

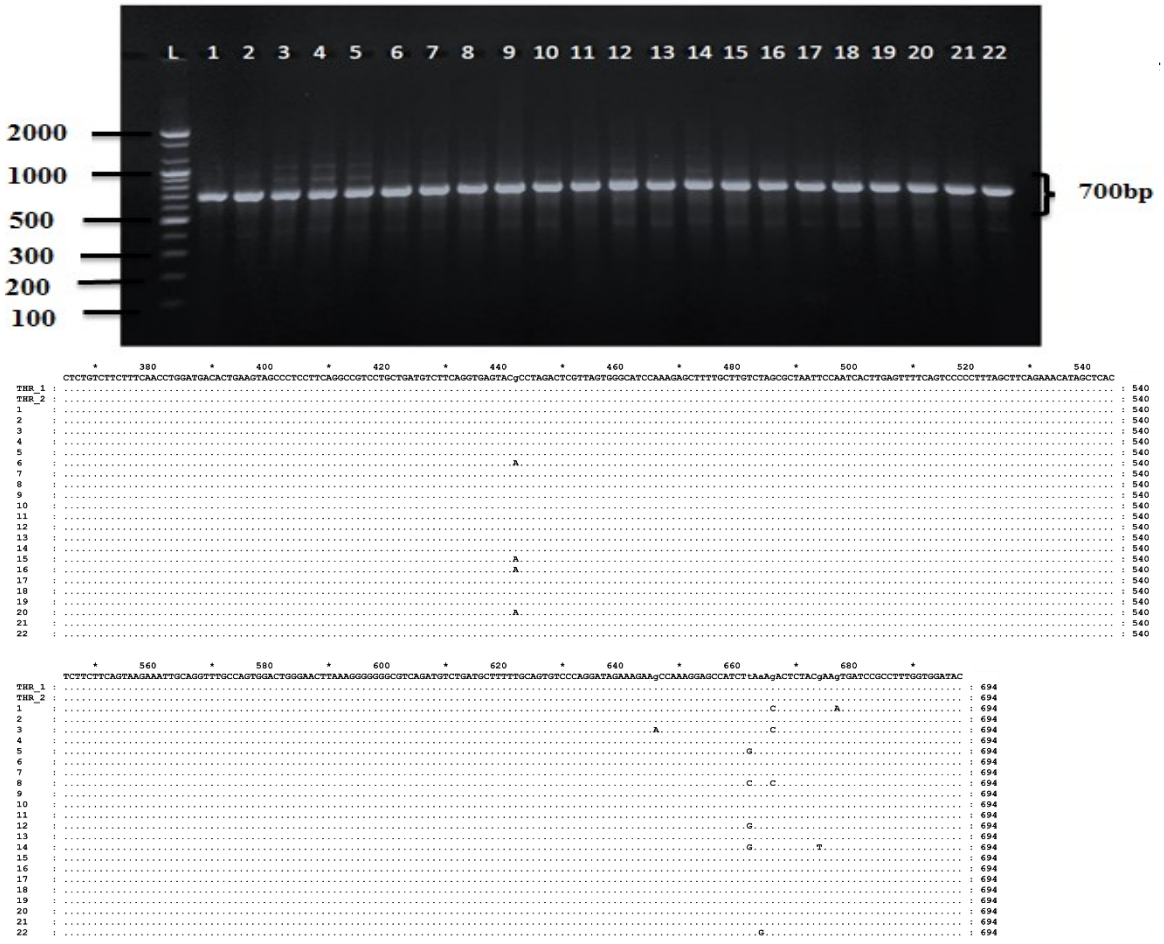

Figure (5) An alignment of human THRB nucleotide sequences. THRB_1 (NG_009159.1) and THRB_2 (AC093927) represents reference sequences obtained from the NCBI website

4.3.3.3. Haplotype network of *THRB* gene

The current study of the phylogenetic network showed the presence of ten haplotypes in the *THRB* gene, as shown in Table 4, Figure 6.

Four haplotypes in patients with hypothyroidism, six haplotypes in patients with hyperthyroidism, and one haplotype in controls were identified.

Haplotype_1 includes the sample recorded in the National Center for Biotechnology Information (NCBI), which are THRB_1 (NG_009159) and the study sample (control), while Haplotype_2 includes the sample recorded in the National Center for

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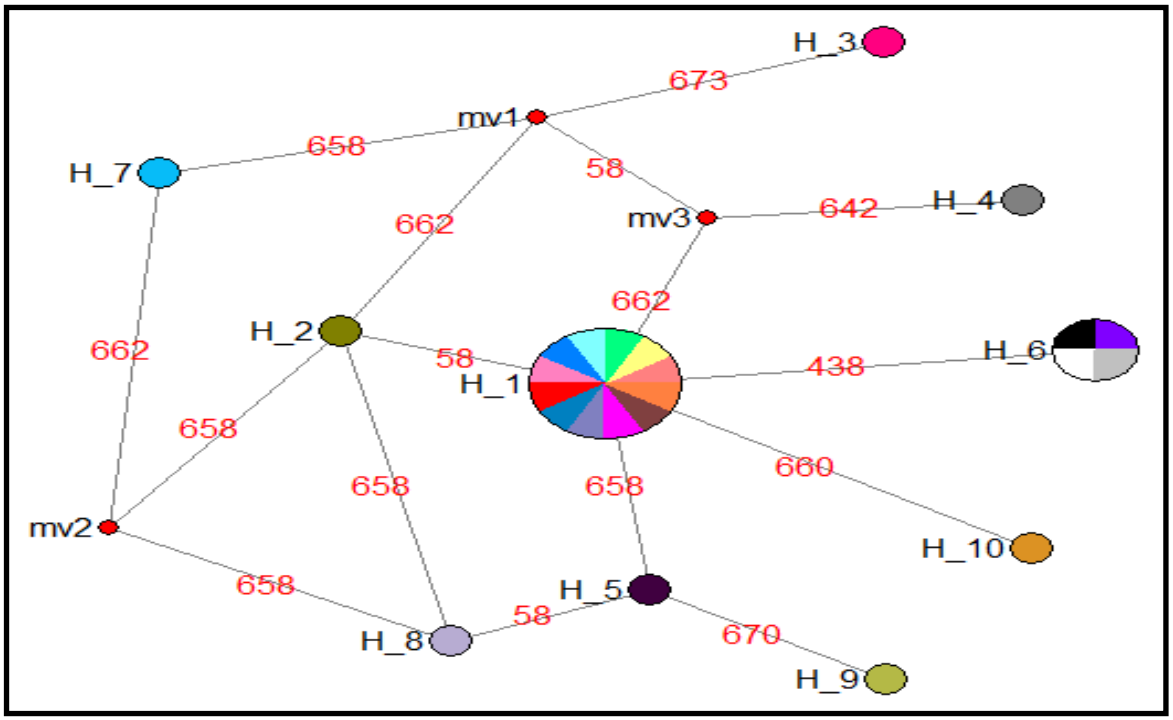
Biotechnology Information (NCBI), which are THRB_2 (AC093927). The other Haplotypes _ 3, 4, 5, 6, 7, 8, 9,10 in this study include the study samples and were not associated with the samples recorded in the NCBI.

Table (4) Haplotypes distribution on study samples and NCBI

	Hypothyroidism	Hyperthyroidism	Control	NCBI
Haplotype_1	-	-	2	THRB_1
Haplotype_2	-	-	-	THRB_2
Haplotype_3	-	1	-	-
Haplotype_4	-	1	-	-
Haplotype_5	-	1	-	-
Haplotype_6	2	2	-	-
Haplotype_7	1	-	-	-
Haplotype_8	1	-	-	-
Haplotype_9	-	1	-	-
Haplotype_10	1	-	-	-



Discussion

The current study did not record any mutation in patients with hypothyroidism, hyperthyroidism, and controls, because most thyroid hormone receptor beta (*THRB*) gene mutations are associated with patients with thyroid hormone resistance syndrome.

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The results of the study did not agree with those of the study conducted by [13]. They diagnosed a missense mutation in exon 11, and the observed amino acid alteration was a substitution of the amino acid valine to methionine at codon 349 (V349M). In addition, the results of the current study did not agree with [14], who found a heterozygous mutation in the *THRB* gene, the missense mutation (c. 1244 G >C) in exon 9, where arginine was substituted by proline. This leads to a change in the structure of thyroid hormone receptor beta; hence, it does not function properly.

In a family with thyroid hormone resistance, the study made by [15] diagnosed a new heterozygous mutation located in exon 9 in the *THRB* gene. Results showed that thyrotropin (TSH) was never suppressed despite the elevated levels of thyroid hormones. The replacement at codon 350 of the polar serine with a non-polar leucine, characterized by a higher molecular weight, is predicted to damage the thyroid receptor function.

Thyroid hormone resistance syndrome (THR) is characterized by inappropriately normal or even elevated thyroid-stimulating hormone levels related to raised levels of free thyroid hormones, free triiodothyronine (FT3), and free thyroxine (FT4) [16, 17]. In percentage of 85 % of the cases, THR is caused by a mutation in the thyroid hormone receptor beta (*THRB*) gene. The mutant receptor exerts a dominant negative influence, leading to a functional impairment [18].

The biologically active thyroid hormone is triiodothyronine (T3), and its actions are mediated by nuclear receptors (TRs), which can bind T3 with high affinity. This hormone-receptor interaction activates or represses specific target genes. All the described mutations in TR-β cause a reduced binding affinity for the ligand (T3). As a result, the mutant thyroid receptors interfere with the activity of the normal thyroid receptor (negative effect), which explains the dominant mode of inheritance of this syndrome [19-21].

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