

The Association of TSHR Gene rs2268458 Polymorphism with Thyroid Disorders (Hyperthyroidism and Hypothyroidism) in a Sample of Iraqi Patients

Yasimin Hassan Juma'a¹, Risala Hussain Allami²

¹Post Graduate; ²Assist Prof. Al-Nahrain University, College of Biotechnology Baghdad, Iraq

Abstract

The Aim: To estimate the association of TSHR gene (rs2268458) polymorphism and some biochemical parameters among hyperthyroidism and hypothyroidism patients

Methods: The study included 100 samples collected from patients who have thyroid disorders (Hyperthyroidism and Hypothyroidism) in The National Diabetes Center, Al- Mustansiriyah University in Baghdad and 50 healthy as a control group. body mass index (BMI), thyroid stimulating hormone (TSH), T3 and T4 assay were performed. The genetic study included an extraction of genomic DNA and PCR technique to examine SNPs polymorphisms (rs2268458) within the TSHR gene in thyroid patients and controls.

Results: The results of nucleotide sequence indicated a statistically significant association between TSHR rs2268458 with hyperthyroidism ($\chi^2 = 4.8$, $P = 0.09$) as compared to healthy group at $P > 0.05$. The mutant homozygous (CC) and allele (C) may be considered as a risk factor for disease ($\chi^2 = 6.86$, $P = 0.008$).

On the other hand, the results of genotype distribution and allele frequency indicated no significant association between TSHR rs2268458 polymorphism and the incidence hypothyroidism ($\chi^2 = 0.78$, $P = 0.7$) as compared to healthy group at $P > 0.05$.

Conclusion: TSHR gene rs2268458 polymorphism was associated with hyperthyroidism and allele C can be a risk factor (OR = 3.3, $\chi^2 = 6.86$, $P = 0.008$) in hyperthyroidism Iraqi patients.

Keywords: Iraqi patients ; Hyperthyroidism; Hypothyroidism; TSHR Gene rs2268458; Thyroid Disorders

Introduction

Thyroid gland requires a sequence of particular conditions to produce its hormones which are affected by a large number of factors. The disturbances of these factors lead to cause thyroid disorders.

Hyperthyroidism disorder also known as "thyrotoxicosis" occurs when the thyroid gland produces excessive amount of thyroid hormone in the blood more than the body needs. Hyperthyroidism causes many of the body's functions to speed up⁽¹⁾. hyperthyroidism linked with a variety of signs as nervousness, fatigue, heat intolerance, trouble sleeping, hand tremors, irregular or rapid heartbeat, diarrhea, weight loss,

goiter⁽²⁾. While Hypothyroidism is known as inability of the thyroid gland to make adequate thyroid hormone to obtain the metabolic requirements of the body⁽³⁾. Also, hypothyroidism linked with a variety of signs as Cold intolerance, Dry skin Fatigue, Difficulty concentrating, Constipation, Depression, Weight gain, Hair thinning/ hair loss, Memory impairment, decreased sweating, slowed heart rate, fertility difficulties or menstrual changes⁽⁴⁾.

Thyroid hormones act a pivotal physiological action on the metabolism and development of the body⁽⁵⁾. Thyroid stimulating hormone receptor (TSHR) plays a crucial role in thyroid hormone metabolism. It is a main controller of function and growth of thyroid cell. TSHR

Mutations may lead to several thyroid diseases (most commonly hyperthyroidism)⁽⁶⁾.

Subjects and Methods

Subjects

The samples were collected from The National Diabetes Center, Al- Mustansiriyah University in Baghdad/Iraq. The patients were diagnosed by a physician which included 50 hyperthyroidism patients, 50 hypothyroidism patients and 50 healthy controls. Five ml of blood sample was collected from the patient antecubital vein was divided into two parts, first part (3 ml) of the patient whole blood was collected in a gel tube and centrifuged for 5 minute at 5000 rpm to separate serum that used for hormonal tests (TSH, T3, and T4) while Second part (2 ml) of the patients whole blood was collected in a EDTA tube For DNA extraction.

Measurements of body mass index (BMI)

Body Mass Index is a measurement of a person's weight (kg) with respect to her or his height (m²).

$$BMI = Weight(kg) / height(m^2)$$

Exclusion criteria

Several pathological, normal physiological conditions and habit can interfere with hyperthyroidism and hypothyroidism cases were excluded from this study such as hypertension, heart disease, diabetes mellitus, smokers and pregnancy.

DNA extraction

For DNA extraction the venous blood, which was collected in the EDTA tubes, was used that was performed using the Geneaid extraction kit (gSYNC™ DNA extraction kit).

PCR:

The primers of TSHR used in this study were designed by Integrated DNA Technologies (IDT) website and based on the data of the National Center for Biotechnology Information (NCBI). The primers sequence and information used in this study were listed in table (1).

Table 1: Sequences of primers used for PCR.

Primer	Sequence (5'.....3')	Tm(°C)	GC%	Product size(bp)
rs2268458- Forwad	CCTCCCTCTTTCCTCCCAGA	59.96	60.00	499
rs2268458- Reverse	TGTTTCCTCTGCATCCCACC	59.96	55.00	

Amplification of the genomic DNA was by the following steps: denaturation at 95°C for 5 minutes, DNA amplification using 30 cycles. Each cycle contains the subsequent steps: denaturation at 94°C for the 1min, annealing for 1min, extension at 72°C for 1min, final elongation at 72°C for 5minutes. The amplified DNA was electrophoresed on 2% agarose with ethidium bromide, (1X) TBE buffer at 70 V for 30 minutes. A 1500 bp ladder was used as a marker for estimating the size of the fragment.

Statistical Analysis

Statistical calculations were made using version

20.0 of the Statistical Package for the Social Sciences (SPSS) program (IBM SPSS Statistics, SPSS Inc., Illinois, Chicago, USA). Continuous variables were expressed as mean (standard deviation) and the differences were accomplished by comparison via student's unpaired 2-sided t-test or one way ANOVA as appropriate. Discrete variables were expressed as counts and frequencies and were compared using the chi-square test. The genotype distributions of SNPs were analyzed in agreement with the Hardy-Weinberg equilibrium. A significant difference is considered at P <0.05.

Results

Anthropometric Analysis (Age and BMI)

In this study, the mean age in the hypothyroidism patient's group was (41.56±13.56) and the mean age in the hyperthyroidism patient's group was (44.58±13.30) while in the control group was (36.48±12.79). There was highly statistical significant difference among the groups as long as the P-value is (0.009). This result agreed with the result of Helmy who documented highly significant difference among hypothyroidism, hyperthyroidism group and control group at $P < 0.05$ (Helmy, 2020)⁽¹¹⁾.

The Biomass index categories showed highly significant differences ($P \leq 0.0005$) between hypothyroidism patients group (25.99±4.754 Kg/m²), hyperthyroidism patients (30.18±6.415 kg/m²) and the healthy control group (30.22±6.814Kg/m²) at ($P \leq 0.05$) these result listed in table (2).

The statistical result of BMI in this study consistent with the results of Makwane *et al.*, (2020)⁽¹²⁾ who found a highly significant differences among the BMI of hypothyroidism, hyperthyroidism patients and the BMI of healthy controls which means there was clear association between increment in BMI (obesity) and the risk of thyroid disorder and its complications.

Table 2 Statistical analysis of Anthropometric parameters for patients and healthy controls.

Variables	Anthropometric parameters			P-value (ANOVA)
	CONTROL (N=50) (mean±SD)	HYPO (N=50) (mean±SD)	HYPER (N =50) (mean±SD)	
Age (Years)	36.48±12.79	41.56±13.56	44.58±13.30	0.009**
BMI (Kg/m ²)	30.22±6.814	25.99±4.754	30.18±6.415	0.0005**
**= difference of high statistical significance, $P \leq 0.05$ †= difference of no statistical significance, $P > 0.05$.				

Table 3 Genetic distribution of TSHR gene polymorphism in the hyperthyroid group and hypothyroid group against healthy controls group.

Genotype	hyperthyroid N (%)	Control N (%)	OR(95%CI)	P-value	X ²	P-value
Hyperthyroidism						
TT	11 (44)	18 (72)	Reference			
TC	7 (28)	5 (20)	2.29 (0.58 -9.02)	0.2†	4.8	0.09†
CC	7 (28)	2 (8)	5.7 (1.004 - 32.67)	0.05*		
T	29 (58)	41 (82)	reference		6.86	0.008*
C	21 (42)	9 (18)	3.3 (1.32- 8.23)	0.01*		

Cont... Table 3 Genetic distribution of TSHR gene polymorphism in the hyperthyroid group and hypothyroid group against healthy controls group.

Hypothyroidism						
TT	16 (64)	18 (72)	Reference		0.78	0.7
TC	5 (20)	5 (20)	1.12 (0.27-4.611)	0.8†		
CC	4 (16)	2 (8)	5.6 (0.25-125.4)	0.2†		
T	37 (74)	41 (82)	reference		0.93	0.3
C	13 (26)	9 (18)	1.6 (0.613- 4.17)	0.3†		

Biochemical Parameters

The result of TSH indicated that the levels were significantly increase (P=0.0001) in hypothyroidism and hyperthyroidism patients in contrast with healthy controls at P≤0.05. The result of the current study agreed with the results of study among the Egyptian population which exhibited a highly significant differences (p=0.001) among hypothyroidism, hyperthyroidism and healthy control groups (Helmy, 2020) Similarly, Bashboosh *et al.*, (2018) a study among Iraqi population showed the same result which they indicated a significant difference in TSH percentages among the three groups (p<0.05) ⁽¹¹⁾ ⁽¹³⁾.

The result of T3 indicated that no significant difference (P=0.2107) among hypothyroidism and hyperthyroidism patients in contrast with healthy controls at P≤ 0.05. The result of this study agrees with

the result of Bashboosh *et al.*, (2018) who described the no significance difference in the T3 concentration among hypothyroidism, hyperthyroidism and healthy control groups. Also, this result is inconsistent with the result of Hamasaeed *et al.*, (2019) who significant relationship in concentrations of T3 in hypothyroidism, hyperthyroidism patients and T3 concentration in healthy controls⁽¹⁴⁾.

The result of T4 indicated that the levels were significantly increase (P=0.005) in hypothyroidism and hyperthyroidism patients in contrast with healthy controls. The result of this study agrees with the result of Hamasaeed *et al.*, (2019) and Taisescu, et al., (2019) whose described the high significance difference in the T4 concentration among hypothyroidism, hyperthyroidism and healthy control groups⁽¹⁴⁾⁽¹⁵⁾.

DNA Extraction

The DNA extraction result was revealed in the figure (1).

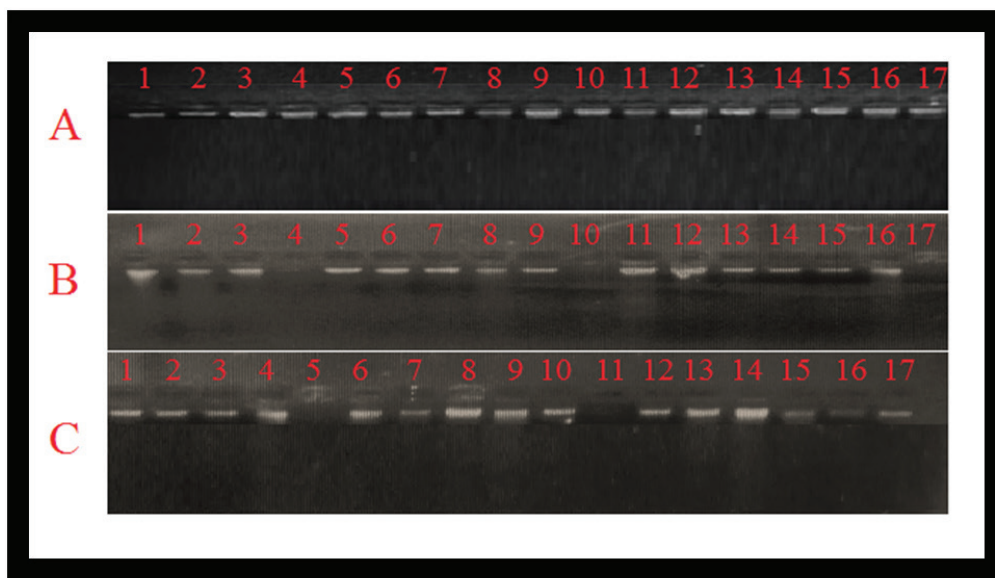


Figure (1): The electrophoresis pattern of genomic DNA extracted from Whole blood cell of (A) healthy controls, (B) hypothyroidism patients and (C) hyperthyroidism patients, 1% agarose at (70V/cm) for 30 min

Amplification of TSHR rs 2268458

The TSHR (rs2268458) gene amplification results showed that the amplification product was 499 bp for all samples as explained in the figure (2).

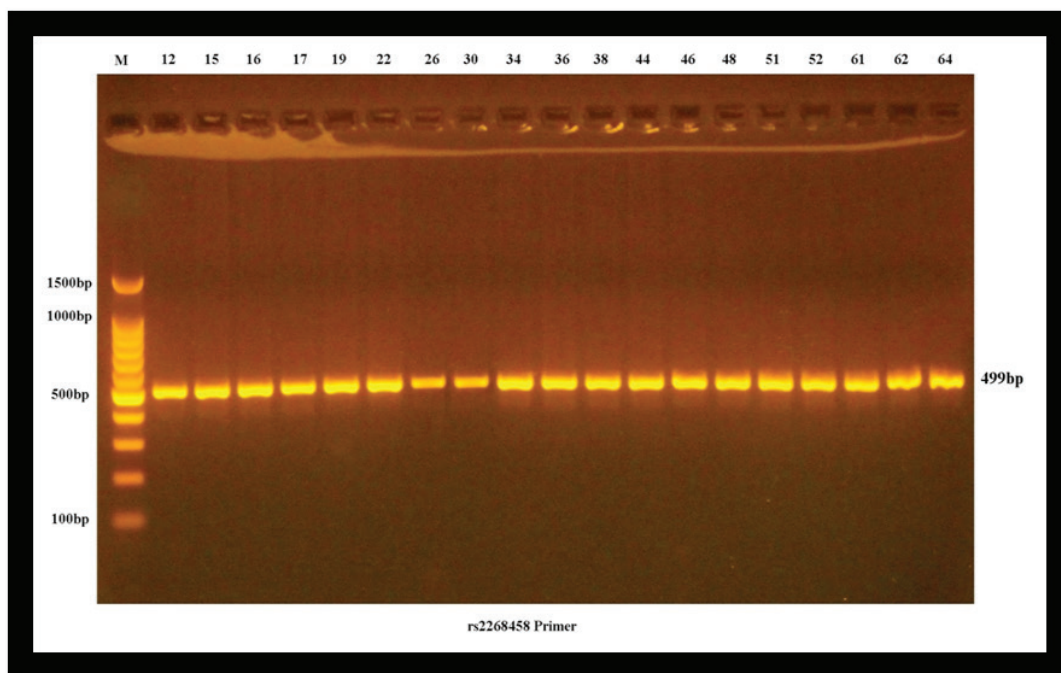


Figure (2): Electrophoresis pattern of PCR product for TSHR gene, amplification product was one band 499bp on agarose gel 2% with DNA ladder 1500 bp at (70V/cm) for 30 min.

Sequence of Amplified thyrotropin Receptor Gene

To study the effect of the genetic variation of TSHR in susceptibility to hyperthyroidism and hypothyroidism in the Iraqi population, the PCR products for rs2268458 was sequenced. Results explained in figure (3) show the position of rs2268458 in the mutated sequence as compared to the wild type of TSHR gene.

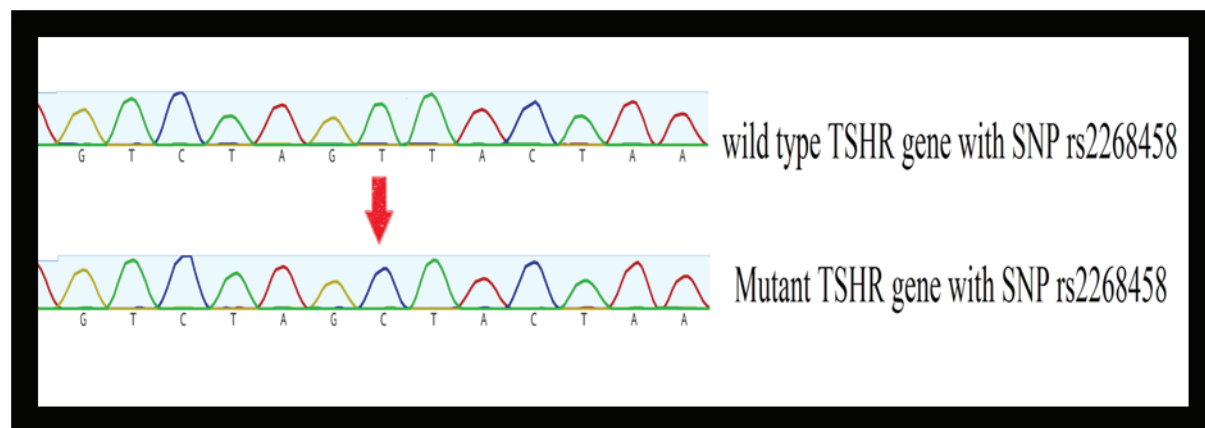


Figure 3-Sequencing chromatograms of TSHR gene polymorphisms (rs2268458)

Frequency and Distribution of TSHR Gene Polymorphism and Susceptibility to hyperthyroidism.

Result in a table (3) documented a significant distribution ($\chi^2 = 4.8$, $P = 0.09$) of rs2268458 genotypes in hyperthyroidism as compared to healthy group at $P > 0.05$. The dominant homozygous genotype (TT) was 44% in Hyperthyroidism and 72% in controls while the mutant homozygous (CC) and the mutant heterozygous (TC) in Hyperthyroidism were 28% and 28% respectively compared to the control which were 8% and 20% respectively. On the other hand, the allele frequency significantly distributed in the two groups ($\chi^2 = 6.86$, $P = 0.008$) in both groups. However, the Fisher's exact test used when expected cell count of more than 25% of cases was less than five and did not exhibited significant difference ($P = 0.09$).

The result of this study agree with the result of Colobran et al., (2011) who described the significance difference in the TSHR (rs2268458) between hyperthyroidism and healthy control groups⁽⁷⁾.

Also, the result in a table (3) documented no significant distribution ($\chi^2 = 0.78$, $P = 0.7$) of rs2268458 genotypes in hypothyroidism as compared to healthy group at $P > 0.05$. The dominant homozygous genotype (TT) was 64% in Hypothyroidism and 72% in controls

while the mutant homozygous (CC) and the mutant heterozygous (TC) in Hypothyroidism were 16% and 20% respectively compared to the control which were 8% and 20% respectively. However, there is no significant difference in the allele frequency between the two groups ($\chi^2 = 0.93$, $P = 0.3$).

this result was consistent with a case-control study between hypothyroidism and healthy controls, there was no significant difference in the TSHR rs2268458 polymorphisms and allele frequency between hypothyroidism group and control group (Al-Azzam et al., 2014)⁽⁸⁾. While this result Inconsistent with the result of case-control study among Iraqi patients with hypothyroidism indicated a significant differences in rs2268458 between hypothyroidism and control groups (Hussain, et al., 2018)⁽⁹⁾.

Yin et al., (2008) demonstrated that the intronic TSHR (rs2268458) was associated with hyperthyroidism but not with hypothyroidism, thus indicating that the TSHR gene has the potential to increase susceptibility to hyperthyroidism that was consistent with our study⁽¹⁰⁾.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: None

Funding: Self-funding

References

- 1- Taylor, P.N., Albrecht, D., Scholz, A., Gutierrez-Buey, G., Lazarus, J.H., Dayan, C.M. and Okosieme, O.E. Global epidemiology of hyperthyroidism and hypothyroidism. *Nature Reviews Endocrinology*, (2018). 14(5), 301.
- 2- Boelaert, K., Torlinska, B., Holder, R.L. and Franklyn, J.A. Older subjects with hyperthyroidism present with a paucity of symptoms and signs: a large cross-sectional study. *The Journal of Clinical Endocrinology & Metabolism*, (2010). 95(6), : 2715-2726.
- 3- Thiagarajan, S., Babu, T.A. and Balaji, R. Subclinical Hypothyroidism in Obese South Indian Children. *The Indian Journal of Pediatrics*, (2019). 86(7), 662-662.
- 4- Chittawar, S., Nagdeote, A., Nair, A., Kawre, K.K. and Dutta, D. Spectrum of clinical symptomatology and its resolution following levothyroxine supplementation in primary and subclinical hypothyroidism: An Indian perspective. *Thyroid Research and Practice*, (2018). 15(1),: 29.
- 5- Mondal, S., Raja, K., Schweizer, U. and Muges, G. Chemistry and biology in the biosynthesis and action of thyroid hormones. *Angewandte Chemie International Edition*, (2016) 55(27), : 7606-7630.
- 6- Stefan, M. and Faustino, L.C. Genetics of thyroid-stimulating hormone receptor—relevance for autoimmune thyroid disease. *Frontiers in Endocrinology*, (2017) 8, : 57.
- 7- Colobran, R., Armengol, M.D.P., Faner, R., Gärtner, M., Tykocinski, L.O., Lucas, A., Ruiz, M., Juan, M., Kyewski, B. and Pujol-Borrell, R. Association of an SNP with intrathymic transcription of TSHR and Graves' disease: a role for defective thymic tolerance. *Human molecular genetics*, (2011). 20(17), 3415-3423.
- 8- Al-Azzam, S.I., Alzoubi, K.H., Khabour, O. and Al-Azzeh, O. The associations of polymorphisms of TSH receptor and thyroid hormone receptor genes with L-thyroxine treatment in hypothyroid patients. *Hormones*, (2014). 13(3), 389-397.
- 9- Hussain, B.I., Hadi, M.A. and AL-Harbi, H.J. The Association of TSHR Gene rs2268458 Polymorphism with Hypothyroidism in Females of Babylon Province-Iraq. *Journal of University of Babylon for Pure and Applied Sciences*, (2018). 26(8), 7-18.
- 10 - Yin, X., Latif, R., Bahn, R., Tomer, Y. and Davies, T.F. Influence of the TSH receptor gene on susceptibility to Graves' disease and Graves' ophthalmopathy. *Thyroid*, (2008). 18(11), 1201-1206.
- 11- Helmy, M.Y. Correlation between Uric Acid and Thyroid Hormones in Patients with Thyroid Disorders. A Case Control Study. *The Egyptian Journal of Hospital Medicine*, (2020). 81(2), 1499-1505.
- 12- Makwane, H., Kare, P.K., Saxena, T. And Jandel, Relationship between thyroid hormones and body mass index in indian healthy adults. *Journal of clinical & diagnostic research*, (2020). 14(5): 15-21
- 13- Bashboosh, N.N., Mohammed, M.H., Najm, R.A. and Kadhim, N.J. Study the Effects of Some Hormonal and Physiological Parameters in Patients with Thyroid Disease. *Journal of Pharmaceutical Sciences and Research*, (2018). 10(6), 1541-1544.
- 14- Hamasaeed, P.A., Hussain, S.K. and Ashraf, S.M. Evaluation of Thyroid Stimulating Hormone and Thyroid Hormone Concentrations in Females with Hypothyroidism and Hyperthyroidism. *Rafidain Journal of Science*, (2019). 28(4), 1-7.
- 15 Taisescu, C.I., Biciusca, V., Sas, L., Camen, G., Sas, T., Albu, C. and Taisescu, O. Changes of visual evoked potentials in patients with thyroid dysfunction. *Rev. Chim.(Bucharest)*, (2019). 10, 961-968.