DOI: 10.24996/ijs.2024.65.1.10

ISSN: 0067-2904

Association of *PARP1* Gene Single Nucleotide Polymorphisms with Papillary Thyroid Carcinoma in The Iraqi population

Reyam J. Alshaikhli[1*], Abdulkareem A. Alkazaz[1] and Abbas A. Aljanabi[2]
[1]*Department of Biotechnology, College of Science, University of Baghdad, Baghdad, Iraq*
[2]*Division of Biotechnology, Department of Applied Sciences, University of Technology, Baghdad, Iraq*

Received: 11/12/2022 Accepted: 25/1/2023 Published: 30/1/2024

Abstract

Thyroid carcinoma incidence is increasing year after year and ranking second among top ten cancers in Iraq, especially among women, and this increased the requirement for ~~the~~ improvement of the molecular detection accuracy because of its potential role in ~~the~~ early detection. Two single nucleotide polymorphisms (rs1136410, A>G and rs1805414, A>G) in *PARP1* gene were found to be associated with thyroid carcinoma risk in several genome wide association studies, therefore, this is a case-control study that was carried out to identify whether these polymorphisms are associated with papillary thyroid carcinoma risk in Iraqi population. The Association was investigated in one hundred and one papillary thyroid carcinoma patients (11 male and 90 female) with ages (22-65), and one hundred and two controls (16 male and 86 female) ~~with~~ ages (21-67), using quantitative PCR-high resolution melting technique. The results showed that there is a strong association between both ~~of~~ rs1136410and rs1805414 with papillary thyroid carcinoma, the allele G was significantly associated with the disease as a risk factor in both variations (p<0.0001, odd ratio(OR): 4.9635, 95% confidence interval(CI): 3.2179-7.6560) in rs1136410, and (P<0.0001, odd ratio(OR): 3.1620, 95% confidence interval (CI): 2.0997-4.7619) in rs1805414. while the allele A represents a protective factor in both variations (OR=0.2015 and 0.3163 respectively). In conclusion, the allele G in both rs1136410 and rs1805414 in the *PARP1* gene is strongly associated with papillary thyroid carcinoma in the Iraqi population.

Keywords: *PARP1*; papillary thyroid carcinoma; case-control study; HRM; polymorphism

علاقة تعدد اشكال النيوكليوتيدات الاحادية لمورث *PARP1* مع سرطان الغدة الدرقية الحليمي في عينة من السكان العراقيين

ريام جميل الشيخلي[1*]، عبد الكريم عبدالرزاق القزاز[1]، عباس عبدالله الجنابي[2]
[1] قسم التقنيات الاحيائية، كلية العلوم، جامعة بغداد، بغداد، العراق
[2] فرع التقنيات الاحيائية، قسم العلوم التطبيقية، الجامعة التكنولوجية، بغداد، العراق

الخلاصة

يتزايد معدل الإصابة بسرطان الغدة الدرقية بشكل مستمر في العراق. وقد وجد بأن تعدد أشكال النيوكليوتيدات الاحادية (rs1136410 A>G, وrs1805414,A>G) للمورث *PARP1* يرتبط بمخاطر الإصابة بسرطان

*Email: reyam.alshekhly@gmail.com

الغدة الدرقية الحليمي في العديد من دراسات الارتباط على نطاق الجينوم، وبالتالي، فهذه دراسة من النوع (المرضى– السيطرة) تهدف إلى تحديد ما إذا كان هذا التنوع مرتبطًا بخطرالاصابة بسرطان الغدة الدرقية الحليمي في المجتمع العراقيين. تم التحري عن هذه العلاقة في مائة وواحد من مرضى سرطان الغدة الدرقية الحليمي العراقيين ومائة واثنين من عينات السيطرة باستخدام تفاعل البلمرة المتسلسل المرتبط بتقنية الانصهار عالية الدقة. أظهرت النتائج أن هناك ارتباطًا وثيقا بين كل من rs1136410 وrs1805414 وسرطان الغدة الدرقية الحليمي، وكان الأليلG لكلا منهما مرتبطًا بشكل كبير بالمرض كعامل خطر (الاحتمالية<0.0003، النسبة الفردية = 2.1014، ثقة الفاصل الزمني 95٪(1.3987–3.1570) في rs1136410 و (الاحتمالية <0.0001، النسبة الفردية 3.1620، ثقة الفاصل الزمني 95٪(2.0997–4.7619) في rs1805414، بينما يمثل الأليل A عامل وقائي لكلاهما (النسبة الفردية=0.2015 و0.3163 على التوالي). تم الاستنتاج بأن الأليل G الموجود في كل من rs1136410 وrs1805414 في المورث*PARP1* يرتبط ارتباطًا وثيقًا بسرطان الغدة الدرقية الحليمي في السكان العراقيين.

1. Introduction

Thyroid carcinoma (TC) is one of the most common endocrine cancers and has a high mortality rate among endocrine neoplasms [1]. Thyroid carcinoma is classified as differentiated thyroid cancer (involving papillary thyroid carcinoma (PTC) and follicular thyroid carcinoma) and undifferentiated thyroid cancer (anaplastic) according to histology [2]. The percentage of 95% of TCs are well differentiated thyroid carcinoma cases (papillary and follicular carcinomas) and when diagnosed early, are mostly associated with a good prognosis and rate of survival [3]. The incidence of TC has increased in the United States and other developed countries rapidly over the past years [4]. In Iraq, in the last few years, TC ranked second among the top ten cancers in women. The incidence trend of the all top ten cancers in females decreased for the last few years, except for ~~the~~ thyroid gland cancer, the incidence trend was increasing [5]. The exposure to radiation is considered the main environmental risk factor, especially at young ages, also the family history of thyroid diseases and having an enlarged thyroid all represent environmental risk factors [6]. The studies of molecular association have identified multiple genes variants for differentiated thyroid carcinoma, and this indicates that there is a high percentage of genetic heterogeneity and the development of this carcinoma tumor represents a multifactorial process in which predisposing genomic variants interact with environmental risk factors which are incompletely understood [7]. If the malignant thyroid was diagnosed early, this allows for achieving a curative thyroid removal. For that, it is very important to distinguish them among benign nodules of the thyroid [8]. For the reason of improving the accuracy in malignancies detection, testing for the mutations of oncogenes has been proposed, which improves the fine needle aspiration diagnosis when it is carried out in indeterminate cytology, ~~that~~ giving support to the clinician's decision. Testing for multiple mutations improves and promotes ~~the~~ performance and specificity [9]. Several genetic factors were found to be associated with thyroid disorders all over the world, such as *LARP7* gene [10] , *CTLA*-4 gene [11], and *FOXE-1* [12]. One of the genes ~~that~~ were found to be associated with thyroid carcinoma is *PARP1*, that expresses the Poly [ADP-ribose] polymerase 1, which is an enzyme belonging to PARP family of enzymes [13]. It is over-expressed in several types of cancers because it is one of six enzymes work on the highly error-prone DNA repairing pathway [14]. Number of studies have been conducted to determine the role of *PARP-1* single nucleotide polymorphisms (SNPs) in susceptibility to thyroid carcinoma in a number of countries such as Belarus [15] and Pakistan [16]. Two single nucleotide polymorphisms (variations each one carried out in a single nucleotide in a specific locus in the genome, which have an important role in the molecular diagnosis) in *PARP1* region (rs1136410 and rs1805414) have been revealed to associate with thyroid carcinoma in a number of ethnics, and because SNPs effects differ from one race to another, and every race should have its ~~own~~ study, and because of the

increasing number of PTC in ~~the~~ Iraq, especially in women, and because there is no published study on the association of rs1136410 and rs1805414 with the PTC in Iraq, this study was conducted.

2. Material and Methods

2.1 Participants recruitment

Informed consent was obtained from both of the PTC Iraqi patients and controls, and all the participants were informed about the study details and consequences, and some personal information was collected from patients and controls using a structured questionnaire. This study was adhered to the tenets of the Declaration of Helsinki and approved by the College of Science Research Ethics committee (Ref.: CSEC/1022/0125) in the University of Baghdad, Iraq and the Iraqi Ministry of Health.

2.2 Blood specimens collection

Blood specimens were collected from 203 participants (101 PTC patients: 90 women and 11 men, of ages (22-65) years who underwent thyroidectomy and were diagnosed and histologically confirmed at the Baghdad Center for Radiation Therapy and Nuclear Medicine, City of Medicine, Baghdad, Iraq according to the criteria of the Iraqi Ministry of Health and 102 controls: 86 women and 16 men, of ages ranged from (21-67) years. The patients' blood specimens were randomly collected from May 2021 to September 2021, while the control samples were collected from unrelated apparently healthy people from various regions in Iraq to be matched with patients in gender and age, who were used to perform a periodic health examination and who did not have a medical history with any type of cancers. The collected blood (5 ml) was divided into two tubes: 3 ml in a serum separation gel tube for the hormonal detection and 2 ml in an EDTA tube for the molecular detection.

2.3 Inclusion and exclusion criteria

The selection criteria was: the patients who were diagnosed with PTC after thyroidectomy, while the cases excluded were: a. Patients with PTC who were treated with radioiodine capsules, chemotherapy, or hormonal therapy. b. Patients with other types of TC (follicular, medullary, and anaplastic), c. Patients with PTC who have a history of cancers other than TC. d. patients with other types of thyroid diseases, and e. Pregnant women.

2.4 Thyroid hormones testing (T3, T4 and TSH)

Three thyroid hormones (T3, T4, and TSH) were investigated in the serum of both ~~of~~ PTC patients and controls in order to evaluate the status of thyroid function using the ELISA technique (TOSOH, Japan).

2.5 DNA extraction

Genomic DNA was extracted using *EasyPure*® Genomic DNA Kit (TransGen Biotech, China) from blood samples conserved in EDTA tubes.

2.6 Quality and quantity analysis of DNA

DNA sample concentrations and purities were estimated by Nanodrop spectrophotometer (Nanovau, China) and gel electrophoresis was carried out for the detection of DNA integrity [17],[18].

2.7 Primers design

The primers were designed using Beacon Designer 8, that are shown in the Table 1 (Bioneer, Korea).

Table 1: Designed primers sequences

Genetic locus	SNP	Primer Sequences (5' – 3')	Annealing Temperature (°C)	HRM (°C)
PARP1 genetic region	**rs1136410**	ADPRT1 **F** ATGTCCAGCAGGTTGTCAAG 20 ADPRT1 **R** GTCTGTCTCATTCACCATGATACC 24	54	Wild 78 mutant 78.5
	rs1805414	ADPRT3 **F** GAGGGCACCGAACACCAT 18 ADPRT3 **R** CATGTTTCTCCACTGGCTGC 20	54	Wild 77.4 mutant 78.1

2.8 DNA genotyping using Qualitative PCR-High Resolution Melting (qPCR-HRM)

All PTC patients and controls in this case-control study were genotyped by using qPCR-HRM technique (Rotor-Gene Q Series Software 2.1.0 QIAGEN, Germany). The specific regions of both ~~of~~ rs1136410 and rs1805414 in *PARP1* gene were amplified using the designed primers (Table 1) and qPCR-HRM conditions (Cycling profile) summarized in Table 3. Amplification reactions were carried out in a total volume of 20 µl containing TransStart® Tip Green qPCR SuperMix (TransGen Biotech, China), 200 nm from each of the forward and reverse primers (Bioneer, Korea), 60 ng of genomic DNA, and the volume was completed to 20 µl using deionized D.W. (Bioneer, Korea). The patient's samples were then compared with control samples

Table 2: Cycling profile of qPCR-HRM

Step	Temperature (^{O}C)	Duration	Cycle no.
Hold	94	1 min.	1
Denaturation	94	5 sec.	35
Annealing	58	15 sec.	
Extension	72	20 sec.	
HRM	60-90	0.1˚C/sec	

2.9 Statistical analysis

Pearson Chi-square test was used to detect the significance which was evaluated based on the minimum *p*-value ($p \leq 0.05$). Hardy-Weinberg Equilibrium (HWE) was used for the comparison between observed and expected values for the evaluation of genotypes distribution in the population. The odd ratios were calculated using the software (https://www.medcalc.org/calc/odds_ratio.php) to check if the alleles and genotypes represent risk factors or protective factors, with confidence of interval (CI estimate at 95%). The statistical analysis was carried out using the IBM SPSS Statistics 22.

3. Results and discussion

One hundred and one PTC patients and one hundred and two control participants have been recruited for this study. The control group samples were collected to be gender matched with the patients group with no statistically significant difference in the distribution of gender between the two groups ($p \leq 0.3$) as shown in Table 3. The percentage of ~~the~~ females suffering from PTC was significantly higher than males in respectively 9:1 and this was suggested to be due to the role of sex hormones in cancer, which is very important and well documented for a number of cancers such as prostate and breast. The effect of sex hormones is mediated by

nuclear receptors which are hormone-specific and regulate gene expression and the biology of tumor cells [19]. The effect of estrogen is mediated by estrogen receptors α- and β- which are expressed in PTC [20]. Researchers found that the polymorphism in estrogen receptors can be a risk factor for TC [21]. The cell proliferation in TC cell lines can adequately increase by estrogen hormone in comparison with male sex hormones [22]. It also modifies the estrogen receptor subtypes expression in TC cell lines. The estrogen increases the levels of estrogen receptor-α in PTC, while there are no significant alterations in anaplastic and follicular thyroid carcinomas, because the effects of estrogen on TC cell lines are type dependent [23]. There is an association between estrogen and ~~the~~ increase in adherence, invasion, and migration in TC cell lines [24]. These observations could play a role in the disparity of gender observed among PTC patients, and can serve as an important and potential target in the treatment of PTC.

Table 3: gender matching between patients and controls groups

Gender	Thyroid carcinoma cases (N=101)		Control (N=102)		Total (%)
	N	%	N	%	
Male	11	10.89	16	15.68	13.30
Female	90	88.23	86	85.14	86.70

Pearson's chi square=1.012; DF=1; p≤0.3(NS)

The mean age ± SD of PTC patients (40.76 ± 10.79) years was higher than controls (37.11 ± 9.72) years, but the difference was not significant ($p \leq 0.08$) as shown in ~~the~~ Figure 1. Papillary

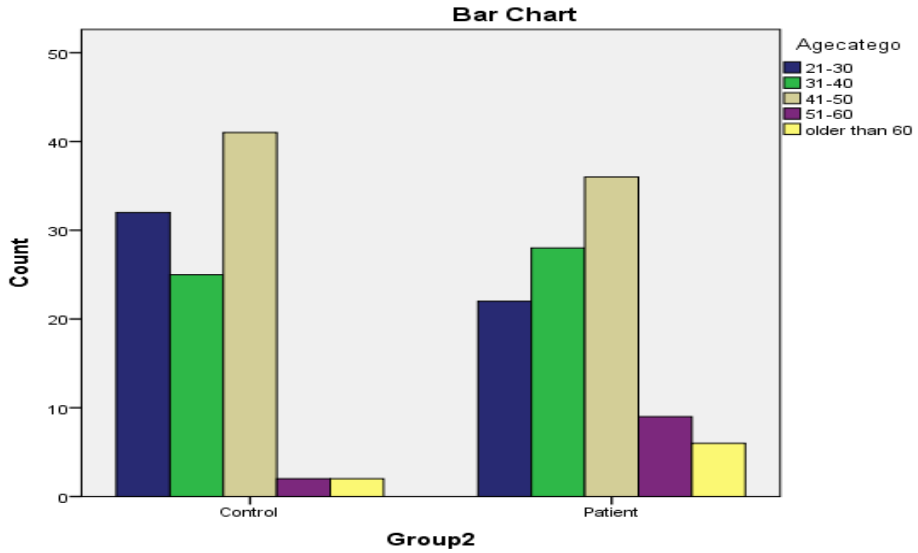


Figure 1: Subjects according to age categories

thyroid carcinoma is popular in various groups of age, but it shows a higher relative frequency in ages between 31 and 50 years [25] and this may be due to that younger individuals own a higher rate of proliferation in the cells of thyroid tissue [26]. The normality test for thyroid hormones revealed that the serum levels of T3 in both ~~of~~ patients and control groups were subjected to a normal distribution, while serum levels of T4 and TSH hormones ~~were~~ significantly deviated from a normal distribution. The results of thyroid hormone levels in patients and controls were shown in ~~the~~ Table 4.

Table 4: Thyroid hormones levels in patients and controls

Group	T3(Mean±SD)	T4Median(range)	TSH Median (range)
Patients	1.39±0.53	89.85±(23.70-139.90)	3.30±(0.71-16.40)
Controls	1.63±0.54	8.10±(7.65-8.30)	3.87±(2.39-4.48)
***p*-value**	0.025	0.009	0.968

The thyroid hormonal levels were altered after surgery, and this come to an agreement with [27] who reported that T4 levels were higher and T3 levels were lower in individuals who underwent ~~a~~ total thyroidectomy in comparison to the control group matched by TSH level. It is suggested that the variable kinetics hypothesis of the thyroid hormonal levels is associated with ~~the~~ thyroid gland manipulation during surgery, but these alterations do not result in clinical effects, including thyrotoxicosis [28]. The differences in the alleles and genotypes frequencies for the patients and controls were tested using HWE and both of them were consistent with HWE with no significant differences for both of the SNPs as shown in ~~the~~ Table 5 and Table 6. The results of the association of alleles and genotypes with PTC revealed that the G allele in rs1136410 was a risk factor for the disease, and its frequency in the patients has a highly significant difference from that in the controls ($p < 0.0001$, odd ratio (OR): 4.9807, 95% confidence interval (CI): 3.2260 to 7.6899), while the A allele in rs1136410 represents a protective factor (OR=0. 0.2008), as shown in ~~the~~ Table 7. Also, the G allele in rs1805414 was a risk factor ($p<0.0001$, odd ratio(OR): 3.1620, 95% confidence interval (CI): 2.0997 to 4.7619), while the allele A also represents a protective factor (OR=0.3163), as shown in ~~the~~ Table 8.

Table 5: Hardy-Weinberg equilibrium (HWE) analysis of SNP rs1136410 in *PARP1* gene among PTC patients and controls.

Genotype	Thyroid carcinoma patients (N=101)		Controls (N=102)	
	N (%)		N (%)	
	Observed	Expected	Observed	Expected
AA	18 (17.82%)	17.47 (17.29%)	64 (62.75%)	61.96 (60.75%)
AG	48 (47.53%)	49.07 (48.58%)	31 (30.39%)	35.07 (34.39%)
GG	35 (34.65%)	34.47 (34.12%)	7 (6.86%)	4.96 (4.87%)
HWE *p* value=	0.8266		0.2408	

Table 6: Hardy-Weinberg equilibrium (HWE) analysis of SNP rs1805414 in *PARP1* gene among PTC patients and controls.

Genotype	Thyroid carcinoma patients		Control	
	N%		N%	
	Observed	Expected	Observed	Expected
AA	14 (13.86%)	18.49 (18.49%)	54 (52.94%)	50.12 (49.14%)
AG	58 (57.43%)	49.02 (49.02%)	35 (34.31%)	42.76 (41.92%)
GG	29 (28.71%)	32.49 (32.49%)	13 (12.75%)	9.12 (8.94%)
HWE *p* value=	0.079		0.066	

The genotypes odd ratio for the heterozygotes (AG) was 0.4264 and the rare homozygotes (GG) odd ratio was 1.4063 for rs1136410, and the odd ratio for the heterozygote (AG) was

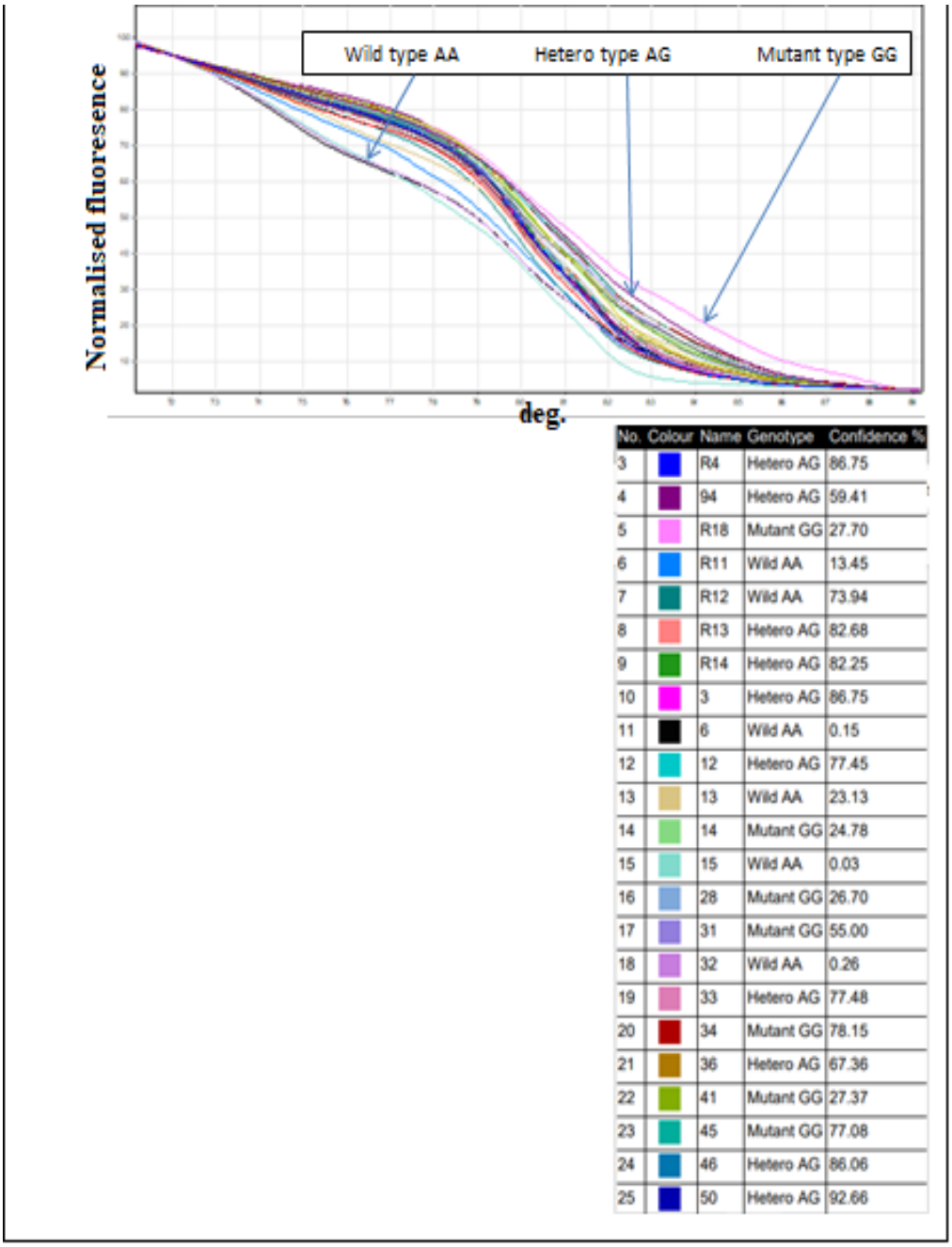
6.3918, while the rare homozygote (GG) odd ratio was 8.6044 for rs1805414. The normalized graph of rs1136410 and rs1805414 generated by qPCR-HRM software is shown in ~~the~~ Figure 2 and Figure 3 respectively. In conclusion, There are strong associations between both ~~of~~ rs1136410 and rs1805414 with PTC in the Iraqi population, and because they represent a risk factor for several other ethnics, additional association studies should be independently carried out for other races because of their significant impact in the molecular diagnosis of the PTC, which is increasing day after day, especially among women in the world, especially with the limitations of the diagnosis and the lack of prognostic factors accuracy, which are important clinical challenges.

Table 7: Association analysis of genotypes and alleles of *PARP1* gene polymorphism rs1136410 with PTC

Allele/ Genotype	Odds Ratio	95% Confidence Interval	*p*-value
A	0.2008	0.1300 to 0.3100	$p < 0.0001$
G	4.9807	3.2260 to 7.6899	$p < 0.0001$
AA	-	-	-
AG	0.4264	0.2134 to 0.8520	$p < 0.0158$
GG	1.4063	0.5355 to 3.6926	$p < 0.4888$

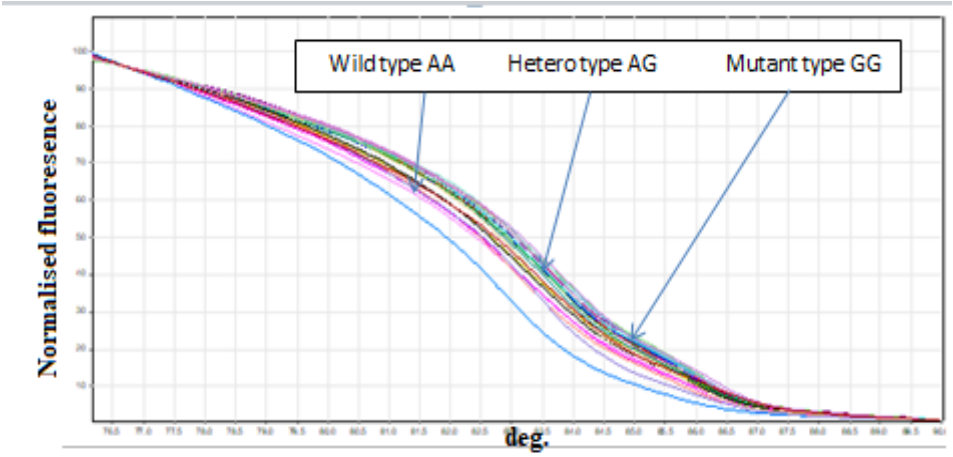
Table 8: Association analysis of genotypes and alleles of *PARP1* gene polymorphism rs1805414 with PTC

Allele/ Genotype	Odds Ratio	95% Confidence Interval	*p*-value
A	0.3163	0.2100 to 0.4763	$p < 0.0001$
G	3.1620	2.0997 to 4.7619	$p < 0.0001$
AA	-	-	-
AG	6.3918	3.1045 to 13.1602	$p < 0.0001$
GG	8.6044	3.5707 to 20.7339	p < 0.0001



No.	Colour	Name	Genotype	Confidence %
3		R4	Hetero AG	86.75
4		94	Hetero AG	59.41
5		R18	Mutant GG	27.70
6		R11	Wild AA	13.45
7		R12	Wild AA	73.94
8		R13	Hetero AG	82.68
9		R14	Hetero AG	82.25
10		3	Hetero AG	86.75
11		6	Wild AA	0.15
12		12	Hetero AG	77.45
13		13	Wild AA	23.13
14		14	Mutant GG	24.78
15		15	Wild AA	0.03
16		28	Mutant GG	26.70
17		31	Mutant GG	55.00
18		32	Wild AA	0.26
19		33	Hetero AG	77.48
20		34	Mutant GG	78.15
21		36	Hetero AG	67.36
22		41	Mutant GG	27.37
23		45	Mutant GG	77.08
24		46	Hetero AG	86.06
25		50	Hetero AG	92.66

Figure 2: Normalized graph of qPCR-HRM profile result of the rs1136410 in *PARP1* gene



No.	Colour	Name	Genotype	Confidence %
3		C37	WilD AA	90.55
4		5b	Hetero AG	88.45
5		C3	WilD AA	51.69
6		2a	Mutant GG	100.00
7		6a	WilD AA	87.10
8		C201	WilD AA	96.43
9		C42	Hetero AG	97.21
10		C200	WilD AA	90.91
11		C49	WilD AA	44.28
12		C214	WilD AA	97.14
13		C32	Hetero AG	82.78
14		C30	WilD AA	96.29
15		3a	WilD AA	89.08
16		C4	WilD AA	73.93
17		C40	WilD AA	93.90
18		C43	WilD AA	61.95

Figure 3: Normalized graph of qPCR-HRM profile result of the rs1805414 in *PARP1* gene

Conclusion

From the obtained results, it can be concluded that there are strong associations between each of the SNPs rs1136410 and rs1805414 with the risk of papillary thyroid carcinoma. The allele G represents a risk factor, while the allele A represents a protective factor, in both of the SNPs.

Acknowledgements

We would like to thank all of the participants and their families for providing this study with blood samples, and the staff members of Baghdad Center for Radiation Therapy and Nuclear Medicine, City of Medicine, Baghdad, Iraq for providing us with the clinical information of papillary thyroid carcinoma patients. We would also like to thank the staff members of the Genetic Engineering Laboratory in the Department of Biotechnology, College of Science, University of Baghdad, Iraq for their excellent technical assistance, and Prof. Dr. Ahmed A. Suleiman, University of Anbar, Anbar, Iraq for designing this study primers and Prof. Dr. Ali

H. Ad'hiah, University of Baghdad, Baghdad, Iraq for helping in statistical analysis and for their kind support and helpful discussion.

Conflict of Interest: The authors declare that they have no conflicts of interest.

References

[1] J. Robbins, M. Merino, J. Boice Jr, E. Ron, K. Ain, H. Alexander, J. Norton, and J. Reynolds. "Thyroid cancer: A lethal endocrine neoplasm". *Ann. Intern. Med.* 115:133-47, 1991.

[2] A. Lam. "Pathology of Endocrine Tumors Update: World Health Organization New Classification 2017 - Other Thyroid Tumors. *AJSP: Reviews & Reports*. 22: 209-16, 2017. https://doi.org/10.1097/PCR.0000000000000183

[3] N. Paricharttanakul, K. Saharat, D. Chokchaichamnankit, P. Punyarit, C. Srisomsap and J. Svasti. " Unveiling a novel biomarker panel for diagnosis and classification of well-differentiated thyroid carcinomas". *Oncol Rep*. 35: 2286-96, 2016. https://pubmed.ncbi.nlm.nih.gov/26782318/

[4] S. Vaccarella, S. Franceschi, F. Bray, C. Wild, M. Plummer and L. Dal Maso. "Worldwide thyroid cancer epidemic? The increasing impact of over diagnosis". *New Eng.l J. Med*. 375:614-617, 2016.

[5] Iraqi Cancer Board. "Annual report Iraqi cancer registry". Iraq: *Iraqi Ministry of Health*. 2016. https://www.jocms.org/index.php/jcms/article/view/1154

[6] T. Carling and R. Udelsman. "Thyroid Cancer". *Ann. Rev. Med*. 65: 125–37, 2014.

[7] E. Bonora, G. Tallini and G. Romeo. "Genetic Predisposition to Familial Non-medullary Thyroid Cancer: An Update of Molecular Findings and State-of-the-Art Studies". *J. Oncol*. 385206. 7p, 2010.

[8] S. Mahar, A. Husain and N. Islam. "Fine needle aspiration cytology of thyroid nodule: diagnostic accuracy and pitfalls". *J. Ayub. Med. Coll.* (Abbottabad). 18:26-9, 2006.

[9] F. Albarel, B. Conte-Devolx and C. Oliver. "From nodule to differentiated thyroid carcinoma: contributions of molecular analysis in 2012". *Ann. Endocrinol*. (Paris). 73:155-64, 2012. https://pubmed.ncbi.nlm.nih.gov/22503804/

[10] I. Ivanovski, S. Giuseppe Caraffi, E. Magnani, S. Rosato, M. Pollazzon, L. Matalonga et al. "Alazami syndrome: the first case of papillary thyroid carcinoma". *J Hum Gen*. 65:133–141, 2020. https://www.researchgate.net/publication/352302847_Clinical_Manifestations_in_a_Girl_with_NAA10-Related_Syndrome_and_Genotype-Phenotype_Correlation_in_Females/download

[11] A. Yassin, A. Al-Kazaz, A. Rahman and T. Ibrahim "Association of *CTLA-4* Single Nucleotide Polymorphisms with Autoimmune Hypothyroidism in Iraqi Patients" *Iraqi J. Sci*. 63,7, 2891-2899, 2022. https://ijs.uobaghdad.edu.iq/index.php/eijs/article/view/5579/2767

[12] R. Alshaikhli, A. Al-Kazaz, A. Aljanabi " Association of rs965513 Polymorphism Near *FOXE1* Gene with Papillary Thyroid Carcinoma in a sample of Iraqi patients " *Iraqi J. Sci.64.12.14, 2023.*

[13] H. Ha and S. Snyder. "Poly(ADP-ribose) polymerase-1 in the nervous system". *Neuro. Dis*. 7 (4): 225–39,2000. https://www.researchgate.net/publication/222214046_PolyADP-ribose__Polymerase-1_in_the_Nervous_System

[14] S. Sharma, S. Javadekar, M. Pandey, M. Srivastava, R. Kumari and S. Raghavan. "Homology and enzymatic requirements of microhomology-dependent alternative end joining". *Cell Death Dis*. 6 (3): e1697, 2015. https://www.nature.com/articles/cddis201558

[15] C. Lonjou, F. Damiola, M. Moissonnier, G. Durand, I. Malakhova, V. Masyakin, F. Le Calvez-Kelm, E. Cardis, G. Byrnes, A. Kesminiene and F. Lesueur. "Investigation of DNA repair-related SNPs underlying susceptibility to papillary thyroid carcinoma reveals *MGMT* as a novel candidate gene in Belarusian children exposed to radiation. *BMC Can*. 2017 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5429528/

[16] K. Bashir, R. Sarwar, I. Mahjabeen, and M. Kayani. " Interaction among susceptibility genotypes of *PARP1* SNPs in thyroid Carcinoma". *PLo. ONE*. 13(9): e0199007, 2018. https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0199007

[17] J. Sambrook, E. Fritsch and T. Maniatis. "Molecular cloning: a laboratory manual. 2nd edition". New York: Cold spring harbor laboratory press, 1989. https://www.scirp.org /(S(i43dyn45teexjx455qlt3d2q))/reference/ReferencesPapers.aspx?ReferenceID=690205

[18] B. Coleman and J. Tsongalis. "Molecular Diagnostics for the Clinical Laboratorian". USA: Humana press. 2005. https://link.springer.com/book/10.1385/1592599281

[19] J.Yager, and J. Liehr. "Molecular mechanisms of estrogen carcinogenesis". *Annu. Rev. Pharmacol. Toxicol*. 36:203–232, 1996.

[20] M. Lee, G. Chen, A. Vlantis, G. Tse, B. Leung and C. van Hasselt. "Induction of thyroid papillary carcinoma cell proliferation by estrogen is associated with an altered expression of *BclxL*. *Cancer J*. 11:113–121, 2005. https://europepmc.org/article/med/15969986

[21] M. Rebai, I. Kallel, S. Charfeddine, F. Hamza, F. Guermazi and A. Rebai. "Association of polymorphisms in estrogen and thyroid hormone receptors with thyroid cancer risk". *J. Recept. Sig. Transduct. Res.* 29:113–118, 2009. https://www.academia.edu/5000300/_Association_of_EGFR_and_HER2_Polymorphisms_with_Risk_and_Clinical_Features_of_Thyroid_Cancer

[22] Q. Zeng, G. Chen, A. Vlantis, and C. van Hasselt. "Oestrogen mediates the growth of human thyroid carcinoma cells via an oestrogen receptor-*ERK* pathway". *Cell Prolif*. 40:921–935, 2007.

[23] Q. Zeng, G. Chen, A. Vlantis, G. Tseand C. van Hasselt. "The contributions of oestrogen receptor isoforms to the development of papillary and anaplastic thyroid carcinomas". *J Pathol*. 214(4):425-33, 2008.

[24] S. Rajoria, R. Suriano, A. Shanmugam, Y. Wilson, S. Schantz, J. Geliebter, et al. "Metastatic phenotype is regulated by estrogen in thyroid cells". *Thyroid*. 20:33–41, 2010. https://pubmed.ncbi.nlm.nih.gov/20067378/

[25] F. Girardi. "Thyroid Carcinoma Pattern Presentation According to Age". *Int. Arch. Otorhinolaryngol*. 21(1): 38–4, 2017.

[26] G. Sassolas, Z. Hafdi-Nejjari, A. Ferraro, M. Decaussin-Petrucci, B. Rousset, F. Borson-Chazot et al. "Oncogenic alterations in papillary thyroid cancers of young patients". *Thyroid*. 22(1):17–26, 2012. https://pubmed.ncbi.nlm.nih.gov/22150560/

[27] M. Ito, A. Miyauchi, S. Kang, M. Hisakado, W. Yoshioka, et al. "Effect of the presence of remnant thyroid tissue on the serum thyroid hormone balance in thyroidecto- mized patients. *Eur. J. Endocrinol*. 173: 333–340, 2015. https://pubmed.ncbi.nlm.nih.gov/26077156/

[28] N. Avenia. "Changes in TSH, T4, T3 and Thyroglobulin Levels throughout Total Thyroidectomy. *J. Clin. Med*. 11(9): 2416, 2022. https://www.ncbi.nlm.nih.gov /pmc/articles/PMC9102100/