

## RESEARCH ARTICLE

# Association of the Asp1312Gly Thyroglobulin Gene Polymorphism with Susceptibility to Differentiated Thyroid Cancer in an Iranian Population

Maryam Abidi<sup>1,2</sup>, Shima Fayaz<sup>2</sup>, Pezhman Fard Esfahani<sup>2\*</sup>

### Abstract

**Background and aim:** While the causes of thyroid cancer in most patients remain largely unknown, it has recently been reported that there may be links to particular chromosome regions. In particular, polymorphisms (SNPs) in the *thyroglobulin (TG)* gene could be susceptibility factors. **Methods:** In this case-control study, any association of the Asp1312Gly single nucleotide polymorphism (SNP) in the TG gene (rs2069556) with susceptibility to differentiated thyroid cancer (DTC) was investigated among 103 Iranian patients and 100 controls who had no history of any type of cancer. Genomic DNA was extracted from the whole blood by salting out procedure. High Resolution Melting (HRM) technique was used to detect this SNP. **Results:** Data were analyzed with SPSS software and the results showed that the recessive GG genotype was associated with an increased risk of differentiated thyroid carcinoma when compared to the AA+AG genotypes (OR: 2.06; CI: 1.09-3.89; P-value: 0.025). **Conclusion:** Although our study demonstrated that differentiated thyroid cancer is significantly associated with this polymorphism, further studies with larger populations are required to confirm our findings.

**Keywords:** High resolution melting (HRM) analysis- Thyroid carcinoma- single nucleotide polymorphism

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### Introduction

Globally, thyroid cancer is the most prevalent malignant tumor of endocrine organs and it is the 7th most prevalent cancer in females, 14th in males and the 11th most frequent malignancy in both genders in Iranian population (Khayamzadeh et al., 2011; Fayaz et al., 2014). Differentiated thyroid cancer includes both papillary thyroid cancer (PTC) and follicular thyroid cancer (FTC) which consists nearly 90% of total malignancies of thyroid gland (Hundahl et al., 1998; Fayaz et al., 2014). The exact reason for the illness still remains unknown. The limited danger elements are including race, and exposure to ionizing radiation in adulthood (Fayaz et al., 2014). Recently, it was demonstrated that the Thyroglobulin (TG) gene is a susceptibility factor for thyroid cancer (Akdi et al., 2011).

Production of TG, a homodimeric glycoprotein of 660 kDa, only occurs in thyroid gland which illustrates a profoundly specialized matrix for the biosynthesis of thyroid hormone (Targovnik et al., 2010). The TG gene is composed of 48 exons, spreading over more than 270 kb on human chromosome 8q24.2–8q24 (Baas et al., 1986; Malthiery and Lissitzky, 1987; Mendive et al., 1999; Moya et al., 2000; Mendive et al., 2001; van de Graaf et al., 2001). TG gene expression is fortified by thyrotrophin (TSH) through the regulation of the intracellular level

of cyclic adenosine monophosphate (cAMP) by means of its receptor (TSHR), situated at the basal layer of the cell (Targovnik et al., 2011). Genetic variables are imperative in thyroid malignancy susceptibility (Akdi et al., 2011). In this case-control study, we tried to figure out any association of Asp1312Gly single nucleotide polymorphism (SNP) in TG gene (rs2069556) with the risk of DTC among Iranian population.

### Materials and Methods

#### Participants

One hundred and three patients referring to Research Institute for Nuclear Medicine of Shariati hospital in Tehran, who had been diagnosed with Differentiated Thyroid Cancer (DTC) by pathology report entered to the study. Similarly, 100 individuals were selected as control with no malignant tumor history. People with a former history of different malignancies, liquor utilization or history of smoking were excluded from the study. Age and sex characteristics of the case and control groups are illustrated in Table 1. All clinical samples were obtained after signing informed consent.

#### DNA extraction

Five ml peripheral blood test was obtained from subjects and collected into tube including EDTA 1 ml

<sup>1</sup>Department of Genetics, Science and Research branch, Islamic Azad University, <sup>2</sup>Department of Biochemistry, Pasteur Institute of Iran, Tehran, Iran. \*For Correspondence: fard-esfahani@pasteur.ac.ir

(1g/dl) and then stored at -20 °C until utilization. DNA was extracted from blood samples by salting out method (Miller et al., 1988).

**PCR-High resolution melting (HRM) analysis**

The evaluation of Asp1312Gly SNP of TG gene was done by Real Time PCR (Corbett Life Sciences-Qiagen). Primers and PCR profile are shown in Table 2. Then, High Resolution Melting (HRM) analysis was done immediately using HRM™ PCR Kit Type-it®, comprising Eva-green fluorescent color. The volume of reactions was 10 µl in 0.1 ml strip tubes, 72-well rotor. The concentration of DNA was about 50 ng/µl in each reaction. PCR-HRM profile was carried out as: Amplification, 95°C for 5 min; 40 × (95°C, 10s; 63°C, 30s; 72°C, 10s); 72°C, 5 min. Heteroduplex enrichment, 95°C for 15s, 60°C for 1 min; HRM, 60–86°C with a 0.1 °C increment per 4 s step.

After normalizing the HRM curves, three different groups of melt curves were observed. In order to determine genotypes of each group, 2 samples from each group were subjected to DNA sequencing analysis.

**Statistical analysis**

Hardy–Weinberg equation for TG (Asp1312Gly) alleles in control groups was done by Chi-square test. Assessment the distinctions in the genotype, haplotype and allele frequency in patients and controls were analyzed by means of Chi-square test. The relationship between polymorphism and DTC risk in each genotypes, were investigated by figuring the crude, age and gender balanced change, odds ratio degree (OR) and corresponding 95% confidence intervals (CIs) utilizing genuine various logistic regression. In haplotype investigation, the most common haplotypes among controls were utilized as reference as a part of the logistic regression model. The p-values reported were based on two-sided likelihood test and considered as significant when were < 0.05. All statistical analyses were carried out with SPSS v13 software.

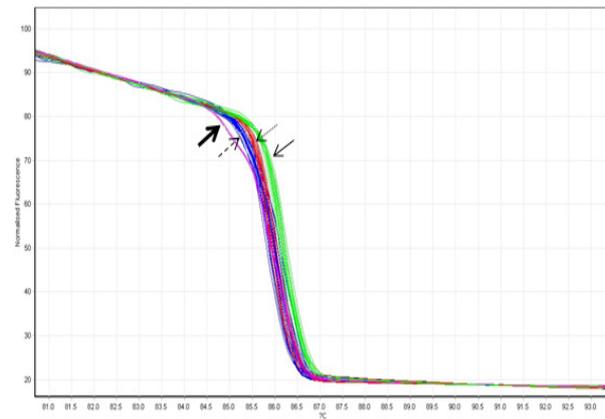


Figure 1. HRM Analysis of Asp1312Gly TGgene in Patients and Controls. Four types of HRM curves were obtained: Solid arrow, Gly/Gly (G/G), dotted arrow, Asp/Asp (A/A) and dashed arrow, Asp/Gly (A/G). (Forth group (thick arrow) includes three samples (one AG genotype, and two AA genotypes)

**Results**

**Characteristics of subjects**

DTC cases as well as controls with no prior or current history of malignant disease were included in the study. All people asserted to have had no past introduction to ionizing radiation sources. General attributes for both groups in every genotype are illustrated in Table 1. There were no statistically differences between DTC patients and control groups in terms of age and gender (Table 1). The frequencies of all polymorphisms in the control populace were in concurrence with the Hardy Weinberg desires.

**HRM analysis**

In HRM investigation, four groups of melting curves were recovered from the people examined. The results of sequencing were aligned with mRNA of TG gene (NM\_003235.4). As expected, the samples ordered in the first group of HRM analysis, showed Asp1312Gly polymorphism in TG gene. The normalized curves of

Table 1. General Characteristics of the Case and Control Groups and the Allelic and Genotype (Asp1312Gly) Frequencies of TG Gene

Variable	Cancer N (%)	Control N (%)	*p value	OR (CI)
Age				
<50	81 (78.6)	88 (88)	0.07	
>50	22 (21.4)	12 (12)		
Sex				
Female	81 (79)	74 (74)	0.43	
Male	22 (21)	26 (26)		
Genotype				
GG	35 (34)	20 (20)		
AG	42 (40.8)	54 (54)		
AA	26 (25.2)	26 (26)		
GG vs AG+AA			0.025	2.06 (1.09-3.89)
G	112 (54.4)	106 (53)	0.417	
A	94 (45.6)	94 (47)		

\*p value<0.05 represents statistical significance

Table 2. Primers, the Amplicon Length and PCR Profile of Asp1312Gly Mutation of TG gene

Mutation	Primers* (5' to 3')	Amplicon size (bp)	PCR Profile
Asp1312Gly	F: CCAGCTGTGGCAGACCATC R: TAACCCTGAGTTGAGTCCAAGCC	202	95 °C, 5 min; cycles (95 °C, 10 s; 63 °C, 30 s; 72 °C, 10 s); 72 °C, 5 min

\*F and R, imply Forward and Reverse primers

<i>Homo sapien</i>	1300	LPPGKMCSADYADLLQTFQVFILDELTARGFCQIQVKTFTGLVSI PVCNNSVQVGGCLTR	1359
<i>Pan troglodytes</i>	1218	LPPGKMCSADYASLLQTFQVFILDELTARGFCQIQVKTFTGLVSI PVCNNSVQVGGCLTS	1277
<i>Gallus gallus</i>	1271	QCDVQKRLWISAAPLYHACQKLQLFQTVQAQTHFQLLLPPQKTCSSDYSGLLQAFQIFIL	1330
<i>Mus musculus</i>	1262	PLICSLESQHWVT-LPPPACQRPQLWQTMQTQAHFQLLLPPGKMCSVDYSGLLQAFQVF	1320
<i>Bos Taurus</i>	1260	EPLLCVQRRR WESRPPQPRACQRPQFWQTLQTAQFQLLLPLGKVC SADYSGLLQAFQV	1319
<i>Macaca mulatta</i>	320	WESQLPQPRACQRPQLWQTIQTQGHFQLQLPPGKMCSADYAGLLQAFQVFILDELTARGF	379
<i>Canis lupus familiaris</i>	1259	GPLVCSLEEGRWLSQPPQACQRPHLWQTFQQTGGFQLQLPPGKMCSADYAGLLPAFQV	1318

Figure 2. Multiple Protein Sequence Alignment of a Selected Region of Homo Sapiens TG with that of Other Species. Highlighted Amino Acids Represent Asp1312 of H. sapiens TG Compared with Other Species.

HRM also represented the differences between first group with GG genotype and the second group with genotype AA and third group with genotype AG (Heterozygote). In addition, the genotypes of all samples discriminated in the fourth group were determined by sequencing (Figure 1). In the fourth group, one of the samples showed AG genotype, while the two other samples showed AA genotype. This discrimination is due to the presence of other SNP(s) reported in this gene.

#### Association analysis

The presence of Asp1312Gly polymorphism of TG gene was assessed by High Resolution Melting (HRM) analysis among patients and controls. The age and sex of cases and controls are demonstrated in Table 1. Chi square test was carried out for the age and sex of two groups utilizing SPSS v13 software and there was no remarkable distinction between age and sex (Table 1). In any case, data showed a noteworthy distinction in TG (AA+AG/GG) genotypes for patients to controls (OR: 2.06; CI: 1.09-3.89; p-value: 0.025) (Table 1).

## Discussion

For the first time, evaluation of Asp1312Gly SNP of TG gene was done by High Resolution Melting (HRM) analysis in order to recognize the predisposition to differentiated thyroid cancer among 203 samples (103 cases and 100 controls).

Genetic factors are vital in susceptibility to thyroid cancer. In a recent report, it was demonstrated that there are relationships between certain chromosome regions and thyroid cancer (Akdi et al., 2011). Recent studies have shown that polymorphisms in Thyroglobulin (TG) gene could be a susceptibility factor for thyroid cancer. In the current study, for the first time, the association of TG Asp1312Gly polymorphism with DTC susceptibility was evaluated. Our findings indicated that there is a relation between the mentioned polymorphism and the risk of DTC.

Figure 2 shows the alignment of human TG protein sequence with other species. As illustrates, human TG Asp 1312 in species such as dog (*Canis lupus familiaris*) and Chimpanzee (*Pan troglodytes*) has existed in the form

of Gly, which may indicate the so called SNP is not fully conserved. Asp1312Gly polymorphism is not located in a specific domain with known function, therefore the association of rs2069556 with DTC could not be easily justified. Also, as far as we know, is not known relevant investigations on this SNP.

Overall, our data demonstrated that the recessive GG genotype was associated with an increased risk of differentiated thyroid carcinoma in comparison with AA+AG genotypes (OR: 2.06; CI: 1.09-3.89; P-value: 0.025). In spite of the fact that our study demonstrated important relationship between this polymorphism and differentiated thyroid tumor, the number of the participants was restricted, and accordingly, further studies with larger populations are needed to confirm these results.

#### Conflict of Interest

The authors do not claim any conflict of interest.

## Acknowledgments

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