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TSHR GENE POLYMORPHISM AND ITS ASSOCIATION WITH HYPOTHYROIDISM IN KASHMIRI POPULATION

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ABSTRACT
Thyroid stimulating hormone receptor gene (THSR) is critical for the normal functioning of the thyroid gland. A mutation in this gene is associated with several disorders of thyroid hormonogenesis including hypothyroidism. In this study, we investigated the role of TSHR codon D727E genetic variants in hypothyroidism. A sample of 445 participants (220 cases and 225 healthy subjects), aged 16-70 years, were randomly selected from among the Kashmiri population. Information on thyroid disease and other lifestyle and environmental exposure were gathered by questionnaire and the patient's endocrinologist or general practitioner verified the diagnosis. The polymorphism of codon D727E of the TSHR gene was identified by polymerase chain reaction-based restriction analysis and direct sequencing. Statistical analysis revealed a single nucleotide polymorphism that is a C-to-G substitution at codon 727 of the TSHR gene (GAC TO GAG). The result showed a 3 fold increased risk among subjects homozygous for G variants as compared to healthy subjects (OR = 3.01; 95% CI = 2.01-4.53; P = < 0.0001). Our study also showed a positive association of the TSHR polymorphism with certain environmental exposures, lifestyle changes, smoking habits and in subjects with high basal metabolic Index (BMI), all of which suggests a possible role of these polymorphisms in the etiology of hypothyroidism in Kashmir.

KEYWORDS: Thyroid stimulating hormone receptor gene, Thyroid peroxidase gene, auto-immune thyroid disorder, Basal Metabolic Index, Goitrogens.

INTRODUCTION
For normal human physiology thyroid gland is very necessary as it plays a crucial role in almost all tissues and influences most functions of the human body through the secretion of its two major thyroid hormones; thyroxine (T_4) and 3-3'-5-tri-iodo thyronine (T_3).[1,2] These hormones control growth, differentiation, regulation of metabolism and other physiological functions in the human body.[3] T_3 is considered to be an active form of thyroid hormone which body can actively use. Whereas, T_4 is more abundant, but an inactive form of thyroid hormone which body cannot utilize and has to be first converted into T_3.[1] Hypothyroidism is a disorder which occurs due to an underproduction of the thyroid hormones T_3 and T_4. It may occur as a result of congenital thyroid abnormalities, AITD (auto-immune thyroid disorders) where the immune system mistakes healthy thyroid tissue as a foreign body and attacks it, such as Hashimoto's thyroiditis[4], the surgical removal of the thyroid gland as a treatment for severe hyperthyroidism and/or thyroid cancer. The prevalence of thyroid disorders as seen throughout the world is 25% in females and 0.6% in males. It has been estimated that about 42 million people in India have thyroid dysfunction.[5] In a hospital based study on the prevalence of thyroid disorders in Srinagar, an overall 40.36% of Kashmir population suffering from thyroid disorders was reported.[6] Subclinical hypothyroidism was found to be the most prevalent thyroid disorder (33.7%) followed by an overt hypothyroidism (5.1%) and hyperthyroidism (1.63%). According to their study, the prevalence of thyroid disorders in males was found to be 36.67% and 41.3% in female subjects. In women, the maximum prevalence of subclinical hypothyroidism was seen in the reproductive age group. The Jammu and Kashmir state is surrounded by mountains and in mountainous areas and hilly region, iodine content are washed due to years washing of the soil by heavy rains and recurrent floods which in turn gives rise to iodine deficient living things which either directly or indirectly

depends on this soil.[7] Diet has also been observed to play an important role in the development of thyroid disorder. The majority of the population of Kashmir valley belong to the Islamic religion and their staple diet is rice, fresh green leafy vegetables and non-vegetarian foods.[8] There are some food types that contain goitrin, thiocyanates and flavonoids called goitrogens, for example, turnip, radish, cabbage, cauliflower, soy foods, etc., which are known to cause goiter and other disorders of the thyroid gland even if there is plenty of iodine in the diet. The consumption of such food items makes it harder for the thyroid gland to absorb iodine by decreasing the activity of the enzyme thyroid peroxidase, which is required to insert iodine into thyroid hormone.[9,10] Previous studies have observed that adult TSH variability is under strong genetic regulation and have estimated a heritability of up to 65%.[11] In patients with atrophic thyroiditis, TSHR blocking antibody binds to the receptor, thereby inhibiting/blocking the binding of TSH to its receptor, thus preventing stimulation of thyroid cell. This results in diminished thyroid hormone output, atrophy of thyroid gland and the clinical state of hypothyroidism.[12] TSHR gene is located on chromosome 14q31, encodes a protein of 764 amino acids. It has a large glycosylated ectodomain of 395 residues which are encoded by 9 exons and the remaining 349 residues encoded by the 10^{th} exon, constitute the seven transmembrane domains and intracytoplasmic tail.[13] One of the diallelic polymorphism of the TSHR gene where a cytosine/guanine base transition takes place at nucleotide position 2281 within codon 727, resulting in the substitution of glutamic acid for aspartic acid (D727E). There are no data for the codon D727E of the TSHR gene polymorphisms among hypothyroidic patients in Kashmir valley. The current study was designed to determine whether the TSHR gene polymorphisms are associated with hypothyroidism in Kashmir study population.

MATERIALS AND METHODS

This study was approved by the Human Research Ethics Committee at the Sheri Kashmir Institute of Medical Sciences and Informed consent was obtained from each subject. The subjects for the current case-control study were 445 adults (220 cases and 225 controls) and included 174 men and 271 women, aged 16-70 years, who were randomly selected from among the Kashmiri population. Subjects with clinically confirmed thyroid disorders (hypothyroidic) were assessed and included as cases in the study while as controls were healthy individuals with no history of any disease/disorder. Subjects with any type of malignancy, diabetes, high cholesterol levels and abnormal lipid profile and those who do not want to participate were excluded. Controls were individually matched to cases in all respects (age ±10 years). Samples were obtained from the community centers and other departments of SKIMS. At study entry, all subjects completed a questionnaire, covering data on demographic, biochemical factors, smoking habits, basal metabolic index including weight and height, type of food intake etc.

Genotypic Analysis

The Genomic DNA was extracted from whole blood samples using modified phenol - chloroform method.[14] The primers for the TSHR gene codon D727E along with their PCR cycling conditions, restriction enzymes, and restriction digestion fragments are listed in table 1. PCR was performed in a total volume of 25µl carried out in 0.2ml PCR tubes (axygen). The PCR reaction mixture consisted of 50-100ng of genomic DNA templates. 200µM of deoxynucleotidetriphosphate [dNTPs] (Biotools), 0.5µM of each primer (Fermentas), 2.5mM $MgCl_2$ and 2.0U of Taq Polymerase with 2.5µl 10x reaction buffer (Biotools). The 265 bp PCR product of TSHR gene codon D7272E was digested with 1 unit of N1AIII (Fermentas) and the reaction buffer according to the manufacturer's instructions. The reaction mixture was incubated for 3 hours at 37^{o}C. The digested product was then resolved on 3% agarose gel (Sisco Research Lab Pvt. Ltd) containing ethidium bromide and evaluated using a gel doc system (AlphaimagerTM 2200, Alpha Innotech Corporation). (Fig. A & Fig. B).

The PCR-RFLP results obtained from the study gene were validated by direct sequencing of PCR products (Sequence scanner software BioEdit was used for comparing sequences between the original PubMed gene sequence) (Fig. C).

Statistical Analysis

The allelic frequencies were estimated by gene counting and genotypes were scored. The odds ratios (ORs) and 95% confidence intervals (CIs) were calculated by using a Yates' continuity corrected chi-square (χ^2) test Statistical analysis of the data was performed using Graph Pad Prism version 6.0 software. The criterion for statistical significance was defined as $P < 0.05$.

RESULTS

In this study, a total of 220 thyroid disorder cases and 225 healthy controls from Kashmir were recruited. These include 81 men and 139 women for cases and 93 men and 132 women for controls. The frequency of cases and controls was found to be higher in females (63.18 vs. 58.66) as compared to males (36.81 vs. 41.33) and in the age group of 27-37 years old (31.81% and 32.44%) respectively. Overweight/obese subjects with an increased basal metabolic index (BMI) were seen to be at higher risk of developing hypothyroidism. Subjects who consume goitrogenic food like turnip, cauliflower, soy, etc. were also observed to be at higher risk of developing hypothyroidism as compared to those who do not consume such food item/s. The screening of the TSHR gene resulted in 3 genotypes; CC, CG, GG at locus 727of the TSHR gene among the recruited subjects with the frequency of 51.8%, 9.3% and 29.5% as seen in subjects with hypothyroidism respectively. In healthy subjects, the frequency of these genotypes was 76.4% (CC), 9.7%

(CG) and 13.7% (GG) respectively. The frequency of D727E variant genotypes (CG and GG) was significantly higher in the hypothyroidic group as compared to the healthy control group (P = 0.0001). The allelic frequency of C allele was 61.13% in cases and 81.3% in controls. Whereas, the allelic frequency of variant allele G was 38.8% in cases and 18.6% in controls (O.R = 2.72, 95% CI = 2.04 - 3.75, P = 0.0001). The distribution of the TSHR D727E genotypes and allelic frequencies between hypothyroidic cases and healthy control subjects are shown in table 2.

Analyzing the effect of the TSHR genotypes by stratifying it against potential variables such as gender, age, dwelling, smoking status, goitrogenic food consumption, etc. Statistically significant results were obtained between the TSHR gene polymorphism and age group of 16-59 years old, both genders, smoking, goitrogenic food consumers, high basal metabolic index, urban dwelling. On the contrary, no statistically significant results were obtained for 60-70 years old age group, subjects with low basal metabolic index and other important factors determining predisposition to this type of thyroid disorder. Correlation assay between TSHR genotypes D727E and its potential variables are shown in Tables 3 & 4.

Table 1: List of Primers, PCR conditions, restriction enzymes, and restriction digestion fragments of the genes of interest.

Gene	Primers	PCR conditions	RE*	AP[a] & DP[b] (bp[c])
TSHR	FP-5′-CATTCCTCTATGCTATTTTCAC-3′ RP-5′-CGTTTGCATATACTCTTCTG-3′	95°C - 60 sec 50°C - 60 sec 35 cycles 72°C - 60 sec	N1a III	AP= 265 W= 160, 84, 21 H= 181,160,84 M= 181, 84

**RE - Restriction enzyme used.*
[a]AP - Amplified products, [b]DP - Digested products, [c]bp - base pairs.
W - Wild allele, H - Heterozygous mutant, M - Mutant allele.

Table 2: Genotypic and allelic frequencies of TSHR gene codon D727E between *hypothyroidic* cases and healthy controls.

Genotypes	Cases N=220 (%)	Controls N=225 (%)	*χ^2	[a]OR (95%CI)[b]	P value
Wild (CC)	114 (51.8)	172 (76.4)	-	Referent	-
Heterozygous (CG)	41 (9.3)	22 (9.7)	12.30	2.81 (1.60 - 4.97)	0.0005
Mutant (GG)	65 (29.5)	31 (13.7)	21.28	3.16 (1.94 - 5.16)	< 0.0001
Variants (CG+ GG)	106 (48.18)	53 (23.5)	8.31	3.01 (2.01 - 4.53)	< 0.0001
C allele	269 (61.13)	366 (81.3)	-	Referent	-
G allele	171 (38.8)	84 (18.6)	43.41	2.72 (2.04 - 3.75)	< 0.0001
Total	440	450			

a; OR - Odds Ratio.
b; CI - Confidence Interval.
**; χ^2 - Chi square test.*

Table 3: Genotypic and allelic frequencies of TSHR gene among *hypothyroidic* cases and controls stratified by different age groups.

Genotypes/Variables	Cases N=220 (%)	Controls N=225 (%)	*χ^2	OR[a] (95%CI)[b]	P value
16 - 26 years age group					
Wild (CC)	26 (11.8)	41 (18.2)	-	**Referent**	-
Heterozygous (CG)	9 (4.0)	3 (1.3)	4.03	4.73 (1.17-19.11)	0.04
Mutant (GG)	2 (9.0)	7 (3.1)	8.22	4.50 (1.67- 12.14)	0.004
CC+GG	11 (5.0)	10 (4.44)	0.71	1.73 (0.64-4.65)	0.40
27 -37 years age group					
Wild (CC)	33 (15.0)	57 (25.3)	-	Referent	-
Heterozygous (CG)	12 (5.4)	10 (4.4)	1.66	2.07 (0.80 - 5.32)	0.19
Mutant (GG)	25 (11.3)	6 (2.6)	16.15	7.19 (2.67-19.35)	<0.0001
CC+GG	37 (16.81)	16 (7.11)	13.37	4.0 (1.93-8.26)	0.0003
38 - 48 years age group					
Wild (CC)	29 (13.1)	45 (20.0)	-	Referent	-
Heterozygous (CG)	9 (4.0)	5 (2.2)	2.08	2.79 (0.85-9.17)	0.14
Mutant (GG)	8 (3.6)	2 (0.8)	4.41	6.20 (1.23-31.32)	0.03
CC+GG	17 (7.72)	7 (3.11)	6.07	3.76 (1.39-10.21)	0.01

49 - 59 years age group					
Wild (CC)	15 (6.8)	21 (9.3)	-	**Referent**	-
Heterozygous (CG)	10 (4.5)	2 (0.8)	4.70	7.0 (1.33-36.70)	0.03
Mutant (GG)	10 (4.5)	10 (4.4)	0.10	1.40 (0.46-4.20)	0.74
CC+GG	20 (9.1)	12 (5.33)	2.17	2.33 (0.88-6.19)	0.14
60 - 70 years age group					
Wild (CC)	11 (5.0)	8 (3.5)	-	Referent	-
Heterozygous (CG)	1 (0.45)	2 (0.8)	0.03	0.36 (0.02-4.74)	0.86
Mutant (GG)	2 (0.90)	6 (2.6)	1.30	0.24 (0.038-1.53)	0.25
CC+GG	3 (1.36)	8 (3.55)	1.54	0.27 (0.05-1.36)	0.21

a; OR - Odds Ratio.
b; CI - Confidence Interval.
**; χ^2 - Chi square test.*

Table 4: Genotypic and allelic frequencies of TSHR gene among *hypothyroidic* cases and healthy controls stratified by gender, smoking, resident, Basal metabolic Index (BMI), goitrogenic food intake, family history, boiled water intake.

Genotypes/Variables	Cases N=220 (%)	Controls N=225 (%)	$^{*}\chi^2$	OR[a] (95%CI)[b]	P value
Gender (Male), n = 81					
Wild (CC)	53 (24.09)	77 (34.2)	-	**Referent**	-
Heterozygous (CG)	19 (8.6)	10 (4.4)	3.0	2.25 (0.97-5.18)	0.08
Mutant (GG)	09 (4.09)	6 (2.6)	1.32	2.18 (0.73- 6.48)	0.25
CC+GG	28 (12.72)	16 (7.11)	6.02	2.54 (1.25-5.15)	0.01
Gender (Female), n = 139					
Wild (CC)	61 (27.7)	95 (42.2)	-	**Referent**	-
Heterozygous (CG)	22 (10.0)	15 (6.6)	4.26	2.28 (1.10 - 4.74)	0.04
Mutant (GG)	56 (25.4)	22 (9.7)	20.94	3.96 (2.20-7.14)	<0.0001
CC+GG	78 (35.45)	37 (16.44)	20.73	3.28 (1.97-5.45)	<0.0001
Smoke (never), n = 142					
Wild (CC)	79 (35.9)	108 (48.0)	-	Referent	-
Heterozygous (CG)	20 (9.1)	7 (3.1)	8.37	3.90 (1.57-9.69)	0.003
Mutant (GG)	43 (19.5)	22 (9.7)	10.10	2.67 (1.48-4.82)	0.0017
CC+GG	63 (28.6)	29 (12.8)	15.94	2.97 (1.75-5.03)	<0.0001
Smoke (Moderate), n = 58					
Wild (CC)	25 (11.3)	45 (20.0)	-	**Referent**	-
Heterozygous (CG)	17 (7.7)	5 (2.2)	10.10	6.12 (2.01-8.60)	0.0015
Mutant (GG)	16 (7.2)	7 (3.1)	6.73	4.14 (1.50-1.34)	0.0009
CC+GG	33 (15.0)	12 (5.33)	14.04	4.95 (2.17-11.26)	0.0002
Smoke (Heavy), n = 20					
Wild (CC)	10 (4.5)	19 (8.4)	-	Referent	-
Heterozygous (CG)	04 (1.8)	10 (4.4)	0.001	0.76 (0.19-3.05)	0.96
Mutant (GG)	06 (2.7)	2 (0.8)	2.70	5.70 (0.96-33.6)	0.10
CC+GG	10 (4.54)	12 (5.33)	0.25	1.58 (0.50-4.93)	0.61
Resident (Rural), n = 86					
Wild (CC)	56 (25.4)	85 (37.7)	-	Referent	-
Heterozygous (CG)	17 (7.7)	10 (4.4)	4.08	2.58 (1.10-6.04)	0.04
Mutant (GG)	13 (5.9)	11 (4.8)	1.21	1.80 (0.75-4.28)	0.27
CC+GG	30 (13.6)	22 (9.7)	4.26	2.07 (1.08-3.94)	0.03
Resident (Urban), n = 134					
Wild (CC)	58(26.3)	87 (38.6)	-	Referent	-
Heterozygous (CG)	24 (10.9)	12 (5.3)	7.23	3.0 (1.39-6.47)	0.007
Mutant (GG)	52 (23.6)	20 (8.8)	18.72	3.9 (2.11-7.20)	< 0.0001
CC+GG	76 (34.5)	32 (14.2)	21.71	3.56 (2.10-6.05)	<0.0001
Basal Metabolic Index (Underweight), n = 7					
Wild (CC)	05 (5.0)	3 (3.5)	-	Referent	-
Heterozygous (CG)	02 (0.45)	2 (0.8)	0.042	0.60 (0.053-6.80)	0.80
Mutant (GG)	0 (0.90)	0 (2.6)	-	Nil	-

CC+GG	02 (0.9)	2 (0.8)	0.04	0.60 (0.05-6.80)	0.83
Basal Metabolic Index (Normal), n = 134					
Wild (CC)	74 (33.6)	107 (47.5)	-	Referent	-
Heterozygous (CG)	25 (11.3)	15 (6.6)	1.30	0.091 (0.003-2.35)	0.24
Mutant (GG)	35 (15.9)	19 (8.4)	5.34	2.41 (1.2-4.88)	0.02
CC+GG	60 (27.2)	34 (15.1)	12.14	2.55 (1.52-4.27)	0.0005
Basal Metabolic Index (Overweight), n =79					
Wild (CC)	35 (15.9)	62 (27.5)	-	Referent	-
Heterozygous (CG)	14 (6.3)	5 (2.2)	7.73	4.96 (1.64-14.93)	0.005
Mutant (GG)	30 (13.6)	9 (4.0)	17.0	5.90 (2.51-13.85)	<0.0001
CC+GG	44 (20.0)	14 (6.2)	21.42	5.56 (2.68-11.56)	<0.0001
Goitrogenic Food Intake (Yes), Cases = 93, Controls = 212					
Wild (CC)	50 (22.7)	166 (73.7)	-	Referent	-
Heterozygous (CG)	19 (8.6)	17 (7.5)	12.17	3.71 (1.80-7.67)	0.0005
Mutant (GG)	24 (10.9)	29 (12.8)	9.37	2.74 (1.46-5.14)	0.002
CC+GG	43 (19.5)	46 (20.4)	17.67	3.10 (1.84-5.23)	<0.0001
Goitrogenic Food Intake (No), Cases = 127, Controls = 13					
Wild (CC)	64 (29.1)	6 (2.6)	-	Referent	-
Heterozygous (CG)	22 (10.0)	5 (1.7)	0.35	0.51 (0.13-1.99)	0.55
Mutant (GG)	41(18.6)	2 (0.8)	0.17	1.92 (0.37-9.98)	0.68
CC+GG	63 (28.6)	6 (2.6)	0.07	0.98 (0.30-3.21)	0.78
Family History (Yes), Cases = 96, Controls = 56					
Wild (CC)	48 (21.8)	44 (19.5)	-	Referent	-
Heterozygous (CG)	20 (9.09)	5 (2.22)	5.16	3.66 (1.26-10.61)	0.02
Mutant (GG)	28 (12.72)	7 (3.11)	7.0	3.66 (1.45-9.23)	0.007
CC+GG	48 (21.8)	12 (5.3)	11.0	3.66 (1.72-7.80)	0.001
Family History (No), Cases = 74, Controls = 169					
Wild (CC)	46 (21.0)	128 (56.8)	-	Referent	-
Heterozygous (CG)	21 (9.54)	17 (7.5)	10.70	3.43 (1.66-7.08)	0.001
Mutant (GG)	07 (3.18)	24 (10.66)	0.05	0.81 (0.32-2.01)	0.81
CC+GG	28 (12.7)	41 (18.2)	4.02	1.90 (1.05-3.41)	0.04
Boiled Water Intake (Yes), Cases = 138, Controls = 150					
Wild (CC)	68 (5.0)	128 (3.5)	-	Referent	-
Heterozygous (CG)	28 (0.45)	9 (0.8)	19.9	5.85 (2.61-13.12)	<0.0001
Mutant (GG)	42 (0.90)	13 (2.6)	28.6	6.08 (3.05-12.10)	<0.0001
CC+GG	70 (31.8)	22 (9.7)	41.34	5.98 (3.41-10.51)	<0.0001
Boiled Water Intake (No), Cases = 82, Controls = 75					
Wild (CC)	46 (5.0)	44 (3.5)	-	Referent	-
Heterozygous (CG)	13 (0.45)	13 (0.8)	0.015	0.95 (0.40-2.30)	0.9
Mutant (GG)	23 (0.90)	18 (2.6)	0.11	1.22 (0.58-2.56)	0.73
CC+GG	36 (16.3)	31 (13.7)	0.02	1.11 (0.59-2.10)	0.87

a; OR - Odds Ratio.
b; CI - Confidence Interval.
**; χ^2 - Chi square test.*

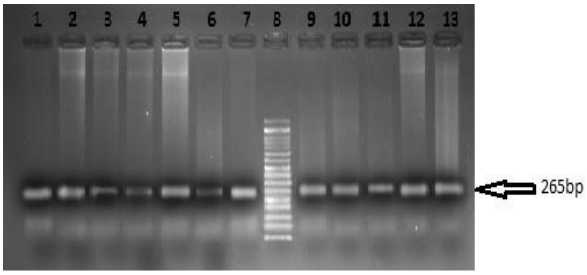

Figure A: Representative gel picture (1.5%) showing 265 bp PCR product of TSH-R gene, lane 1-25 represent amplicon of codon 727 and lane M (50bp) represents molecular weight marker.

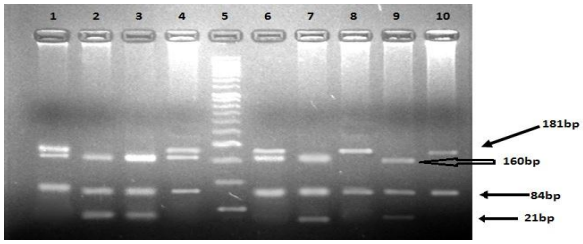

Figure B: Representative gel picture showing RFLP analysis of TSH-R gene Codon 727: Lane no.1, 4 and 5 - CG (heterozygous variant genotype), Lane no. 2, 3, 6 and 8 – CC (wild/normal genotype) and Lane no. 7 and 9 - GG represents homozygous mutant genotype. Lane M is the DNA size marker (50bp).

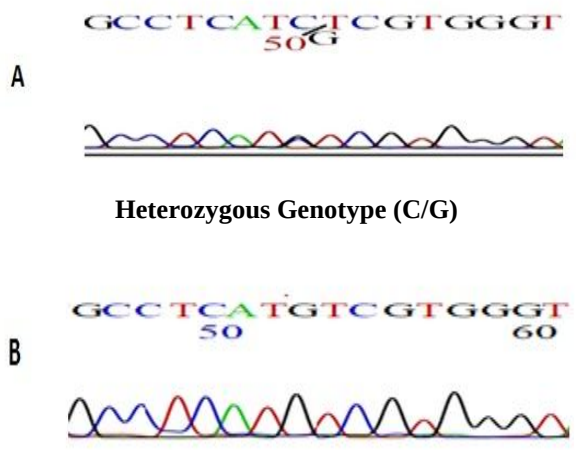

Figure C: Chromatogram of *TSHR* genotype codon D727E showing; (A): C/G transition and (B): C/C genotype.

DISCUSSION

Thyroid disorders comprise a considerable portion of the study in endocrine disorders, other than diabetes mellitus.[15] However, the clinical behavior of the thyroid is substantial in most diseases and act as a cornerstone for diagnosis and therapy. This study demonstrates the association between two genetic variations in the TSHR gene (Thyroid stimulating hormone receptor) and enhanced hypothyroid susceptibility. The outcomes indicated that there may be an increased risk of developing hypothyroidism in the presence of some alleles in the studied polymorphism. This is the first study showing that the TSHR gene codon D727E exon 10 variant genotypes are associated with an increased risk of thyroid disorders in a Kashmiri population. Subjects with high intake of goitrogenic food (e.g., turnip, soy, rabbi, etc.) were also seen to be at an increased risk of developing hypothyroidism. All the subjects (patients as well as healthy group) in our study were non-vegetarian, i.e., their main diet was red meat, fish, chicken, etc. According to a survey on the prevalence of thyroid disorder induced by demography and food habits in a South Indian population; the relative risk of developing a thyroid disorder is quite high in non-vegetarian population and excessive intake of iodine rich food can also lead to auto-immune thyroid disorder.[5] Another research article reported that a high-fiber diet can cause fluctuation of thyroid hormones.[16] Three germ-line mutations of the TSHR gene have been identified and described in a population; codon D36H, codon P52T in the extracellular portion and the third one is D727E within the intracellular portion of the receptor.[17] Our study focused on the codon D727E of thyroid stimulating hormone gene (TSHR). Variant genotypes of TSHR gene codon D727E showed an increased association with hypothyroidism separately as well as when analyzed with respect to a different lifestyle and environmental exposures. We obtained a statistically significant result for TSHR D727E polymorphism with respect to age, gender, smoking status, dwelling, goitrogenic food intake and high basal metabolic index. When subjects were analyzed based on their smoking patterns; non-smokers and moderate smokers (2-3 cigarettes per day) were observed to be at the significantly higher risk of developing hypothyroidism. This observation is consistent with the previous observations[18,19] in which a correlation between smoking and hypothyroidism was found. Higher risk of hypothyroidism among smokers may also be attributed to an increase in serum thiocyanate concentration from smoking. In our study, a positive association was found between higher basal metabolic Index (BMI) and TSHR polymorphism suggesting that this SNP could have a role in the development of this disorder and is also known to be a risk factor for coronary heart disease.[20-22] Few studies on the codon D727E of TSHR gene confirmed that the effect of TSH can be inhibited by inhibitory TSHR antibodies and the TSH dependent signaling is primarily induced via the TSHR - the α subunit of the stimulatory G protein ($G_S\alpha$) adenylate cyclase-cAMP pathway.[23] Several mutations which have been found in this TSHR gene can be categorized into germline or acquired mutations and germline loss-of-function mutations. Germline loss-of-function TSHR mutations are associated with TSH resistance and congenital hypothyroidism.[24] In several Caucasian population, the TSHR D727E polymorphism was seen to be associated with lower levels of plasma TSH but not with FT4 which may be due to the higher sensitivity of the variant as compared to the wild-type TSHR as less TSH is needed to produce normal FT4 levels.[25-27] A significant association of D727E polymorphism exon 10 of the TSHR gene was also reported in a cohort study done on patients with congenital hypothyroidism.[28] The significant association could represent a common genetic predisposition factor for the development of thyroid disorder in subjects. Our study is consistent with the studies that inactivating mutations of TSHR gene can account for several cases of hypothyroidism. Therefore, a better understanding of the normal variation in TSH at genetic level may improve the sensitivity of its use in its screening and help provide a better understanding of the thyroid profile and disorders in humans. Analysis of these variations in TSH levels and the genes responsible may be particularly important in a population at risk for abnormal TSH levels.

CONCLUSION

This is the first observational study to examine the association between the single nucleotide polymorphism (SNP) of the TSHR gene and risk of hypothyroidism in Kashmir valley. Our data suggest an elevated risk for this particular thyroid disorder in individuals with the codon 727 TSHR polymorphism, suggesting a possible role for this gene polymorphism in the etiopathogenesis of hypothyroidism in our valley. In particular, this study emphasized the effect of different lifestyle and environmental factors, e.g. age, sex, smoking status,

residence, food intake, BMI, etc. as the potential confounders which could modify the association between TSHR gene SNP and hypothyroidism.

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COMPETING INTERESTS

The authors declare that there were no competing interests.

REFERENCES

1. Kirsten, D. The thyroid gland: physiology and pathophysiology. *Neonatal network*: NN, 2000; 19(8): 11-26.
2. Panicker V, S.G Wilson, J.P Walsh, J.B Richards, S.J Brown, J.P Beilby, A.P Bremner, et al. "A locus on chromosome 1p36 is associated with thyrotropin and thyroid function as identified by genome-wide association study." *American journal of human genetics*, 2010; 87(3): 430-435.
3. Andersen S., Pedersen, K.M., Bruun, N.H., Laurberg, P. Narrow individual variations in serum T(4) and T(3) in normal subjects: a clue to the understanding of subclinical thyroid disease. *J Clin Endocrinol Metab*, 2002; 87: 1068-72.
4. www.medicinenet.com. emedicine Health. Retrieved on 02-07; www.medicinenet.com, & on, M.R., (2010). Thyroid Disorder Information, 2010.
5. Nimmy, N.J., Aneesh, P.M., M.P. Udupi, R.H., Binu, K.M. A survey on the prevalence of thyroid disorder induced by demography and food habits in South Indian Population. *Indian Journal of Pharmacy Practice*, April-June, 2012; 5(2).
6. Rama, Jailkhani., Shivashankara, A. Ramachandrayya., Vidya, S. Patil., Sameena. A hospital-based study of prevalence of thyroid dysfunction in Srinagar, Jammu and Kashmir state of India. *Int J Med Sci Public Health*, 2015; 4(2): 151-154. doi: 10.5455.
7. Chaturvedi, S., Sanjay, M., Pushpa, Gupta, and et al. Assessment of Iodine induced Disorders. *JIMA*, 2006; 94: 127-35.
8. Lidhoo, M.L. Kashmiri Tribes. Srinagar, India: *Meenakshi Publishers*, 1992; 7.
9. Chandra, A.K. Iodine nutritional status of school children in rural area of Howrah district in west Bengal. *Indian J. Physcol Pharmacol*, 2004; 48(2): 219-24.
10. Abigail Gelb Fruzza, Carla Demeterco-Berggren, Kenneth Lee Jones. Unawareness of the Effects of Soy Intake on the Management of Congenital Hypothyroidism. Pediatrics, September, 2012; 130(3). doi:10.1542/peds.2011-3350.
11. Panicker, V, Wilson, S.G, Spector, T.D, Brown, S.J, Falchi, M, Richards, J.B., et al. Heritability of serum TSH, free T4 and free T3 concentrations: a study of a large UK twin cohort. *Clin Endocrinol* (Oxf), 2008; 68: 652-9. 7.
12. Manorama Swain, Truptirekha Swain and Binoy Kumar Mohanty. Autoimmune Thyroid Disorders - An Update. *Indian Journal of Clinical Biochemistry*, 2005; 20*(1):* 9-17.
13. Ren-De, Liu., Rui-Xiong, Chen., Wen-Rui, Li., Yu-Liang, Huang, Wen-Hu, Li., Guang-Rong, Cai., Heng, Zheng. The Glu727 Allele of Thyroid Stimulating Hormone Receptor Gene is Associated with Osteoporosis. *North American journal of Medical Sciences*, July, 2012; 4(7): 300-4.
14. Sambrook, J.R.D. Molecular cloning: *A laboratory manual*, 2001.
15. Sartorius, N., Ustun, T,B., Lecrubier,Y and Wittchen, H,U. Depression co- morbid with anxiety: results from the WHO study on psychological disorders in primary health care. *Br J Psychiatry*, 1996; 177: 38-43.
16. Hitman, S., Kelly, F.C. Prevalence of congenital hypothyroidism. *Indian Journal of Endocrinology*, 1999; 45(4): 245-9.
17. Duprez, L., Parma, J., Van, Sande, J., et al. Germline mutations in the thyrotropin receptor gene cause non-autoimmune autosomal dominant hyperthyroidism. *Nat Genet*, 1994; 7: 396-401.
18. Wilmar, M, Wiersinga. Smoking and thyroid, *Clinical Endocrinology*, August 2013; 79(2): 145–151.
19. S, Fukata, K., Kuma, M, Sugawara. Relationship between cigarette smoking and hypothyroidism in patients with Hashimoto's thyroiditis. *Journal of Endocrinological Investigation*, October 1996; 19(9): 607–612.
20. Al-Azzam, S,I., Alkhateeb,, AM., Al-Azzeh, O., Alzoubi, K..H., Khabour, O,F. The Role of Type II Deiodinase Polymorphisms in Clinical Management of Hypothyroid Patients Treated with Levothyroxine. *Exp Clin Endocrinol Diabetes*, 2013; 121: 300-305.
21. Ruhla, S., Weickert, M,O., Arafat, A,M, et al. A high normal TSH is associated with the metabolic syndrome. *Clin Endocrinol (Oxf),* 2010; 72: 696-701.
22. Arnaud-Lopez, L., Usala, G., Ceresini, G., et al. Phosphodiesterase 8B gene variants are associated with serum TSH levels and thyroid function. *Am J Hum Genet*, 2008; 82: 1270-1280.
23. Drvota, V., Janson, A., Norman, C., *et al*. Evidence for the presence of functional thyrotropin receptor in cardiac muscle. *Biochem Biophys Res Commun*, 1995; 211: 426-31.
24. Abramowicz, M.J, Duprez, L., Parma, J., Vassart, G., Heinrichs, C. Familial congenital hypothyroidism due to inactivating mutation of the

thyrotropin receptor causing profound hypoplasia of the thyroid gland. *J Clin Invest*, 1997; 99: 3018-3024.

25. Van, Der, Deure, W.M., Uitterlinden, A.G., Hofman, A. Rivadeneira. F., Pols, H.A., Peeters, R.P., et al. Effects of serum TSH and FT4 levels and the TSHR-Asp727Glu polymorphism on bone: the Rotterdam Study. *Clin Endocrinol (Oxf)*, 2008; 68: 175-81.
26. Peeters, R.P., Van, Der, Deure, W.M., Van, Den, Beld, A.W, Van, Toor, H., Lamberts, S.W., Janssen, J.A., et al. The Asp727Glu polymorphism in the TSH receptor is associated with insulin resistance in healthy elderly men. *Clin Endocrinol (Oxf)*, 2007; 66: 808-15.
27. Hansen, P.S., Brix, T.H., Sorensen, T.I., Kyvik, K.O., Hegedüs, L. Major genetic influence on the regulation of the pituitary-thyroid axis: a study of healthy Danish twins. *J Clin Endocrinol Metab*, 2004; 89: 1181-7.
28. Maslinda, Musa., Fatimah, Harun and Sarni Mat, Junit. An investigation into the D727E polymorphism in the *TSH receptor* gene sin patients with congenital hypothyroidism. *AsPac J. Mol. Biol. Biotechnol*, 2008; 16*(3):* 65-69.