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Research Article

Association between MTHFR Gene Polymorphisms (C677T, A1298C) and Subclinical Hypothyroidism Susceptibility

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Abstract

Introduction: Methylenetetrahydrofolate Reductase (MTHFR) is a key enzyme for f olate and homocysteine metabolism. Two common polymorphisms of MTHFR gene (C677T and A1298C) reduce the MTHFR activity by various degrees. This study is aimed to investigate the association between MTHFR gene polymorphisms and Subclinical Hypothyroidism (SCH) in the Georgian population.

Materials and Methods: 87 patients with SCH and 87 age-matched healthy controls were enrolled in this study. Methylenetetrahydrofolate reductase C677T and A1298C polymorphisms in the patient and control groups were evaluated using the polymerase chain reaction - restriction fragment length polymorphism (PCR-RFLP) method.

Results: A significant difference was observed in frequency of both 677CT and 677TT genotypes between SCH and control groups (for CT 40% vs. 17%; OR = 3.89, 95% CI: 1.90 – 7.96, p<0.001; For TT 12% vs. 2%; OR = 8.33 95% CI: 1.74–39.88, p<0.001). Similarly, T allele frequency was significantly higher in the SCH group compared to the healthy controls (36.1% vs. 10.9%; OR = 3.84, 95% CI: 2.16 – 6.81, p<0.001). No difference in genotypic and allelic distributions was observed between SCH and control groups for the MTHFR A1298G polymorphism.

Conclusions: The data indicate that the MTHFR C677T polymorphism, but not A1298G, is a significant risk factor for the subclinical hypothyroidism in Georgian population.

Keywords: Subclinical Hypothyroidism; MTHFR; SNPs

Introduction

Hypothyroidism is a clinical condition in which the thyroid gland does not produce enough thyroid hormones, most often due to thyroid failure (primary hypothyroidism) [1]. Overt hypothyroidism is diagnosed when serum Thyroxine levels (T4) are lower than the reference range. In overt hypothyroidism due to thyroid dysfunction, Thyroid-Stimulating Hormone (TSH) levels are appropriately elevated. The population reference range of TSH is around 0.4–4.5 mIU/L and most patients with overt hypothyroidism have TSH above 10 mIU/L [2]. Subclinical Hypothyroidism (SCH) is defined as mild elevation of thyroid-stimulating hormone levels when serum thyroid hormone levels are within normal reference range [3]. Approximately 90% of patients with subclinical hypothyroidism have serum thyrotropin levels lower than 10mU/L [4].

Homocysteine (Hcy) is a sulfur-containing amino acid naturally found in human blood. Increased circulating levels of Hcy or Hyperhomocysteinemia (HHcy) is recognized as an independent risk factor for cardiovascular disease [5,6]. Additionally, decreased folate and increased plasma Hcy levels are associated with a variety of common conditions such as neural tube defects [7], strokes [8], Alzheimer's disease [9], Parkinson's disease [10], certain types of cancer [11], osteoporosis [12], insulin resistance [13], Gastrointestinal disorders [14], Down's syndrome [15], and cleft palate [16]. Plasma Hcy concentration was reported to be elevated in hypothyroidism and subclinical hypothyroidism patients [17-20].

Methylenetetrahydrofolate Reductase (MTHFR) is an enzyme that plays a central role in folate and Hcy metabolism by catalyzing the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, the primary circulatory form of folate which is utilized in Hcy remethylation to methionine [21]. Also, Hcy can be considered as an intermediate in the S-Adenosyl Methionine (SAM) cycle. SAM is a universal methyl-group donor for methylation of a wide variety of biological substrates [22].

The gene for 5,10-methylenetetrahydrofolate reductase or the MTHFR gene is located on the short (p) arm of chromosome 1 (1p36.22) [23,24]. The MTHFR gene has been identified to possess two common variants, rs1801133 (C677T) and rs1801131(A1298C). The C to T transition at nucleotide 677 in exon 4 of the MTHFR gene results in an Alanine (A) to Valine (V) substitution (A222V). Individuals with homozygous variant 677TT and heterozygote 677CT genotypes have only about 30% and 65%, respectively, of in vitro enzyme activity as compared to those with 677CC wild type. The

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frequency of C677T polymorphism varies in different populations [25-27]. Another common SNP is A to C change in 1298 position (A1298C) at exon 7 causing glutamate to alanine substitution on the 429th position, resulting the decreased MTHFR activity, which is more pronounced in the homozygous than heterozygous state. Neither homozygosity nor heterozygosity is associated with higher plasma Hcy or a lower plasma folate concentration [28].

To our knowledge, few studies have addressed the association between MTHFR genotypes and the risk of thyroid disorders [29-31].

In this study we investigated associations between MTHFR variants and mild thyroid failure defined as a Subclinical Hypothyroidism (SCH) which have not been previously studied.

Materials and Methods

Subjects

A total 174 individuals were enrolled in this study (87 patients with SCH and 87 age-matched healthy controls). Patients were recruited in this study from the National Institute of Endocrinology and medical center "Medimedi" (Tbilisi, Georgia) between 2016 and 2019. The study protocol was approved by the Ethics Committee of Tbilisi State Medical University. Written informed consent was obtained from all patients and controls. Detailed information on medical history was obtained from all study subjects. Patients inclusion criteria provided in a Table 1; Patients with known history of autoimmune thyroiditis were excluded. Controls (n = 87) which showed no evidence of thyroid disorder were recruited. Baseline demographic data and a medical history were obtained from each control subject. Clinical and demographic characteristics of subjects are given in Table 2.

MTHFR genotyping

Venous blood was collected from patients and healthy subjects in K3EDTA coated tubes (5ml). DNA extraction was performed using QIAamp DNA Mini Kit (Qiagen, Maryland, USA). MTHFR gene polymorphisms were detected by Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RELP) method. Primers, PCR conditions and Restriction enzymes provided in Table 3. The PCR product of C677T (198-bp band) was digested using HinfI restriction enzyme (NEB, USA), fragments were separated by 3% agarose gel electrophoresis and then visualized using UV Transilluminator (Enduro, USA). After digestion, fragment sizes for the C677T variant were: 198-bp bands for CC, 175, 23-bp bands for TT, and 198, 175, and 23-bp bands for CT.

The PCR product of A1298C (128-bp band) was digested using MboII restriction enzyme. The resulting fragments were separated by 3% agarose gel electrophoresis and then visualized using UV Transilluminator (Labnet Int., NJ, USA). The digestion fragment sizes for the A1298C variant were: 72 and 28-bp bands for AA, 100 and 28-bp bands for CC, and 100, 72 and 28-bp bands for AC. As a negative control we used all PCR components except the DNA template in every PCR run.

Statistical analysis

Genotype and allele frequencies for the MTHFR genotype variants were investigated using standard Chi-square (χ^2) analysis. Odds Ratios (OR) and 95% confidence intervals (95% CI) were calculated for association between MTHFR genotypes/alleles and subclinical hypothyroidism. A p value < 0.05 was considered statistically significant. Data was expressed as mean ± Standard Deviation (SD). The analysis was performed using SPSS software version 23 (Chicago, IL).

Table 1: Patients inclusion criteria.

inclusion criteria				
Adults > 20				
Male and Female				
Upon diagnosis level of TSH≤15 mIU/L				
Level of FT4 - Normal range				
Anti-thyrotropin receptor antibody (TRAb) - Negative				
Anti-thyroglobulin antibody (TgAb) – Negative				
Dietary supplements use (folate, B6 and B12) - No				

Table 2: Clinical and demographic characteristics of patients and controls at the time of sampling.

Variables	Case	control
Age (mean ± SD)	45.2 ± 12.2	44.6 ± 14.1
n (female/male)	87(82/5)	87(80/7)
TSH mIU/mL (N 0.35-4.94)	11.4±2.67	2.82±0.75
Anti-TPO ab IU/mL (N < 5.61)	Negative	-
Anti-TG ab IU/mL (N < 4.11)	Negative	-
Current treatment	L-thyroxine	None

Data are expressed as mean standard deviation.

Results

87 Subclinical hypothyroidism patients, and 87 age-matched controls were enrolled in this study. Both groups were more likely to be females. The groups of patients and controls did not significantly differ concerning gender or age. Table 4 shows the allele frequencies and genotype distributions for MTHFR C677T and A1298C, and their ORs and 95% CIs for SCH. The genotype frequencies for both polymorphisms were in accordance with the Hardy-Weinberg equilibrium in the case and control groups.

In our study, the T allele frequency distributions of MTHFR were significantly different between patient and control groups (31.6% versus 10.9%, P<0.001; Table 4). The frequencies of the MTHFR C677T genotypes in the patients (CC, 48%; CT, 40%; TT, 12%) were also significantly different from controls (CC, 81%; CT, 17%; TT, 2%) (P<0.001). The MTHFR CT and TT genotypes were significantly associated with an increased risk of SCH (OR = 3.89, 95% CI: 1.90 – 7.96, p<0.001 and OR = 8.33 95% CI: 1.74–39.88, p<0.001, respectively). When we combined heterozygous and homozygous variant genotypes, the OR for the CT+TT genotypes was 4.41; 95% CI: 2.24–8.68, p<0.001. In addition, individuals with the T allele have a significantly higher risk of SCH compared to individuals with C allele (OR = 3.84, 95% CI: 2.16 – 6.81, p<0.001). However, for the MTHFR A1298G polymorphisms, no differences in the frequencies of the genotypes or alleles were seen in the patients and controls (For AC genotype OR = 0.95, CI: 0.42–2.11, P=0.9; For CC genotype OR = 1.52, CI: 0.41–5.64, p=0.53). The frequency of the C allele was not higher in SCH patients compared to healthy controls (OR = 1.15, 95% CI: 0.63 – 2.11, P=0.64).

Table 3: Primers, PCR conditions and restriction enzymes for MTHFR variants.

MTHFR variants	Primer pairs	PCR conditions	Restriction enzymes	
C677T	F/5′TGAAGGAGAAGGTGTCTGCGGGA3′ R/5′AGGACGGTGCGGTGAGAGTG3′	94°C for 5 min (96°C for 30 s, 66°C for 30 s,72°C for 30 s) 30 cycles 72°C for 5 min	HinfI	
A1298C	F/5′CAAGGAGGAGCTGCTGAAGA3′	96°C for 30 s, 61°C for 30 s, 72°C for 30 s) 32 cycles 72°C for 5 min	MboII	
	R/5′CCACTCCAGCATCACTCACT3′			

Table 4: MTHFR C677T and A1298C polymorphisms in patients with SCH and control subjects.

Variables	SCH (n=87)	Control (n=87)	Odds Ratio	*p* value
	n(%)	*n*(%)	(95% CI)	
Genotype or Allele				
C677T				
CC	42 (48.3)	70 (80.5)	Reference	
CT	35 (40.2)	15 (17.2)	3.89 (1.90 – 7.96)	<0.001
TT	10 (11.5)	2 (2.3)	8.33 (1.74–39.88)	<0.001
CT + TT	45 (51.7)	17 (19.5)	4.41 (2.24–8.68)	<0.001
C	117 (67.2)	155(89.1)	Reference	
T	55 (31.6)	19 (10.9)	3.84 (2.16 – 6.81)	<0.001
A1298C				
AA	67 (77)	68 (78.2)	Reference	
AC	14 (16.1)	15 (17.2)	0.95(0.42–2.11)	0.9
CC	6 (6.9)	4 (4.6)	1.52 (0.41–5.64)	0.53
AC+ CC	20 (23)	19 (21.8)	1.07 (0.52–2.18)	0.77
A	148 (85.1)	151 (86.8)	Reference	
C	26 (14.9)	23 (13.2)	1.15 (0.63 – 2.11)	0.64

Discussion

Subclinical hypothyroidism has been estimated to occur in 3.5 per 1,000 women and 0.8 per 1,000 men yearly in the United States [32]. The prevalence of subclinical hypothyroidism varies from 3% to 15% based on the study population. Statistical research demonstrates a higher incidence of subclinical hypothyroidism in women and elderly individuals [33]. In the Republic of Georgia, incidence of different forms of hypothyroidism is 6.11 per 1000, and has estimated nearly 23 000 new cases in 2018 [34].

The risk of progression to overt hypothyroidism from subclinical hypothyroidism is 2 to 6% per year [35]. The management of patients with mildly elevated serum TSH level with levothyroxine is controversial. There is a risk of over-treatment, which could cause iatrogenic hyperthyroidism and negative outcomes including osteopenia and atrial fibrillation [36]. Initiation of early therapy with T4 is not clearly associated with benefit [37]. However, when the TSH level is above 10 mIU/L, levothyroxine therapy is agreed to be appropriate, because of the progression to overt hypothyroidism. In the era of "precision medicine", the decision regarding treatment of SCH should be individualized according to the patient's clinical status [38].

Identification of biomarkers such as serum Homocysteine (Hcy) or folate might be useful for determining treatment initiation of SCH. Even a mild increase in the levels of Hcy is considered a risk factor for several diseases in humans. It has been reported that subclinical hypothyroidism is associated with elevated plasma Hcy concentration [17,18]. On the other hand, The C677T functional variant in MTHFR is a risk factor for hyperhomocysteinemia. Hcy level is higher in MTHFR TT and CT individuals than in CC, while folate level is lower in TT and CT than in CC [39,40]. In addition, the importance of epigenetic mechanisms, such as DNA methylation, in thyroid health has been reported: Genomic DNA was hypomethylated in individuals with Graves' disease [31] and in SCH individuals with MTHFR gene C677T variant [41].

The current study investigated the SCH susceptibility variants of MTHFR gene in Georgian population. Our study indicated that the MTHFR C677T polymorphism is a strong genetic risk factor for subclinical hypothyroidism. When the MTHFR genotype groups were examined by comparing CT and TT genotypes to the homozygous CC genotype it has been demonstrated that the CT and TT genotypes were significantly linked to SCH. Besides, individuals with the T allele have a significantly higher risk of SCH compared to individuals with the C allele. We found that the distributions of genotypes and the C allele frequency for MTHFR A1298C did not significantly differ between the two groups.

These results were contrariwise to previous study suggesting a role for the MHTFR gene as a genetic risk factor for hypothyroidism and hyperthyroidism. The findings of the study suggest that genetic variants of MTHFR at g.1298A>C and its haplotype analysis at 677 and 1298 may modulate the risk of thyroid disorders in Jordanian females [29]. It should be noted that in contrast of previous studies our study was focused on a specific form of thyroid dysfunction defined as a Subclinical Hypothyroidism (SCH). In the present study the selection criteria for patients was as follows: they had a TSH level less than 15 mIU/L (TSH levels were less than 10 mIU/L in approx. 85% of patients) and normal reference range of FT4 upon diagnosis. Rarely TSH level in SCH are more than 10 mU/L [3, 4]. In this setting, during selection of case individuals the diagnosis of SCH was based on anti-thyrotropin receptor antibody (TRAb) and anti-thyroglobulin antibody (TgAb) levels, on a clinical manifestation and in some cases on an ultrasound investigation of the thyroid gland. Elevated TRAb and TgAb served as an exclusion criterion for patients. All individuals from the case group were healthy without clinical or biochemical evidence of intrinsic thyroid disease. Possible mechanisms to explain the associations between MTHFR gene variants and SCH are: First, in subjects with the TT and CT genotypes there is a deficiency of S-Adenosine Methionine (SAM), which is the main donor of a methyl group for DNA [22]. Therefore, hypomethylation of DNA may be involved in the pathophysiology of SCH. Second, elevated plasma Hcy (due to the C677T variant) may directly or indirectly influence on this condition through oxidative stress induced by Hcy [42,43].

The limitations of our study include a relatively small sample size, and difficulties to get data about plasma levels of Hcy or folate, thus we were not able to evaluate the association between blood-

based biomarkers and SCH. However, hyperhomocysteinemia, reduced plasma folate level and their association with an increased risk for many disorders, including vascular and neurodegenerative diseases, autoimmune disorders, birth defects, diabetes, renal disease, osteoporosis, neuropsychiatric disorders, and cancer, was observed in individuals with MTHFR C677T variant [44].

In conclusion, we found that the MTHFR C677T polymorphism is strongly associated with an increased risk of SCH. Early identification of SCH individuals with C677T variants is important for a personalized treatment approach. Vitamin (folate, vitamin B12 and B6) supplementation of such individuals will result in normalization of homocysteine, folate, as well as SAM levels in plasma. This may lead to delay of treatment initiation or the lowering of the levothyroxine dose.

To our knowledge, the association of MTHFR C677T and A1298C variants with SCH has not been previously analyzed and further studies are necessary with a larger sample size to prove our findings. It will also be important for future investigations to focus on how this genetic variant interacts with environmental factors, including stress, diet, alcohol and tobacco consumption, and therapeutic medicine use.

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Disclosure Statement

The authors declared no conflict to disclose.

References

1. Chaker L, Bianco AC, Jonklaas J, Peeters RP. Hypothyroidism. Lancet. 2017; 390: 1550-1562.
2. Aoki Y, Beli RM, Clickner R, Jeffries R, Phillips L, Mahaffey KR. National Health and Nutrition Examination Survey (NHANES 1999-2002). Thyroid. 2007; 17: 1211-1223.
3. Biondi B, Cappola AR, Cooper DS. Subclinical hypothyroidism. a review. 2019; 322: 153-160.
4. Peeters RP. Subclinical Hypothyroidism. N Engl J Med. 2017; 376: 2556-2565.
5. Medina MÁ, Urdiales JL, Amores-Sánchez MI. Roles of homocysteine in cell metabolism old and new functions. Eur J Biochem. 2001; 268: 3871-3882.
6. Nygård O, Nordrehaug JE, Refsum H, Ueland PM, Farstad M, Vollset SE. Plasma homocysteine levels and mortality in patients with coronary artery disease. N Engl J Med. 1997; 337: 230-237.
7. Steegers-Theunissen RP, Boers GH, Trijbels FJ, Finkelstein JD, Blom HJ, Thomas CM, et al. Maternal Hyperhomocysteinemia. A Risk Factor for Neural-Tube Defects. Metabolism. 1994; 43: 1475-1480.
8. McIlroy SP, Dynan KB, Lawson JT, Patterson CC, Passmore AP. Moderately elevated plasma homocysteine, methylenetetrahydrofolate reductase genotype. 2002; 33: 2351-2356.
9. Morris MS. Homocysteine and Alzheimer's disease. The Lancet Neurology. 2003; 2: 425-428.
10. Mattson MP, Shea TB. Folate and homocysteine metabolism in neural plasticity and neurodegenerative disorders. Trends Neurosci. 2003; 26: 137-146.
11. Sun CF, Haven TR, Wu TL, Tsao KC, Wu JT. Serum total homocysteine increases with the rapid proliferation rate of tumor cells and decline upon cell death. A potential new tumor marker. Clin Chim Acta. 2002; 321: 55-62.
12. Herrmann M, Widmann T, Herrmann. Homocysteine–a newly recognised risk factor for osteoporosis. Clin Chem Lab Med. 2005; 43: 1111-1117.
13. Golbahar J, Aminzadeh MA, Kassab SE, Omrani GR. Hyperhomocysteinemia induces insulin resistance in male Sprague. Dawley rats. Diabetes Res Clin Pract. 2007; 76: 1-5.
14. Givvimani S, Munjal C, Narayanan N, Aqil F, Tyagi G, Metreveli N, et al. Hyperhomocysteinemia decreases intestinal motility leading to constipation. Am J Physiol Gastrointest Liver Physiol. 2012; 303: G281-G290.
15. James SJ, Pogribna M, Pogribny IP, Melnyk S, Hine RJ, Gibson JB, et al. Abnormal folate metabolism and mutation in the methylenetetrahydrofolate reductase gene may be maternal risk factors for Down's syndrome. Am J Clin Nutr. 1999; 70: 495-501.
16. Mills JL, Kirke PN, Molloy AM, Burke H, Conley MR, Lee YJ, et al. Methylenetetrahydrofolate reductase thermolabile variant and oral clefts. Am J Med Genet. 1999; 86: 71-74.
17. Morris MS, Bostom AG, Jacques PF, Selhub J, Rosenberg IH. Hyperhomocysteinemia and hypercholesterolemia associated with hypothyroidism in the third US National Health and Nutrition Examination Survey. Atherosclerosis. 2001; 155: 195-200.
18. Nedrebø BG, Ericsson UB, Nygård O, Refsum H, Ueland PM, Aakvaag A, et al. Plasma total homocysteine levels in hyperthyroid and hypothyroid patients. Metabolism. 1998; 47: 89-93.
19. Sengül E, Cetinarslan B, Tarkun I, Cantürk Z, Türemen E. Homocysteine concentrations in subclinical hypothyroidism. Endocr Res. 2004; 30: 351-359.
20. Diekman MJ, van der Put NM, Blom HJ, Tijssen JG, Wiersinga WM. Determinants of changes in plasma homocysteine in hyperthyroidism and hypothyroidism. Clin Endocrinol (Oxf). 2001; 54: 197-204.
21. Rosenblatt DS AL, Sly S, Valle D. Inherited disorders of folate transport and metabolism. In Scriver CR, Beaudet . The Metabolic and Molecular Bases of Inherited Disease. Seventh ed. 1995; 284-289.
22. Selhub J. Homocysteine metabolism. Annu Rev Nutr. 1999; 19: 217-246.
23. Goyette P, Pai A, Milos R, Frosst P, Tran P, Chen Z, et al. Gene structure of human and mouse methylenetetrahydrofolate reductase (MTHFR). Mamm Genome. 1998; 9: 652-656.
24. Frosst P, Blom HJ, Milos R, Goyette P, Sheppard CA, Matthews RG, et al. A candidate genetic risk factor for vascular disease a common mutation in methylenetetrahydrofolate reductase. Nat Genet. 1995; 10: 111-113.
25. Yadav U, Kumar P, Gupta S, Rai V. Distribution of MTHFR C677T gene polymorphism in healthy north Indian population and an updated meta-analysis. Ind J Clin Biochem. 2017; 32: 399-410.
26. Romero-Sánchez C, Gómez-Gutierrez A, Gómez PE, Casas-Gomez MC, Briceño I. gene polymorphism frequency in a colombian population Colomb Med. 2015; 46: 75-79.
27. Yadav U, Kumar P, Rai V. Global prevalence of MTHFR C677T gene polymorphism. A meta-analysis of population based studies. Ind J Clin Biochem. 2014; 29: 123.
28. van der Put NM, Gabreels F, Stevens EM, Trijbels FJ, Eskes TK, van den Heuvel LP, Blom HJ. A second common mutation in the methylenetetrahydrofolate reductase gene an additional risk factor for neural tube defects. Am J Hum Genet. 1998; 62: 1044-1051.
29. Abu-Hassan DW, Alhouri AN, Altork NA, Shkoukani ZW, Altamimi TS, et al. MTHFR gene polymorphisms in hypothyroidism and hyperthyroidism among Jordanian females Arch. Endocrinol. Metab. 2019; 63: 280-287.
30. Chen Y, Wang B, Yan S, Wang YG. Significant association between MTHFR C677T polymorphism and thyroid cancer risk evidence from a meta-analysis. Genet Test Mol Biomarkers. 2014; 18: 695-702.
31. Arakawa Y, Watanabe M, Inoue N, Sarumaru M, Hidaka Y, Iwatani Y. Association of polymorphisms in DNMT1, DNMT3A, DNMT3B, MTHFR and MTRR genes with global DNA methylation levels and prognosis of

autoimmune thyroid disease. Clin Exp Immunol. 2012; 170: 194-201.

32. Gosi SKY, Garla VV. Subclinical Hypothyroidism; Treasure Island. StatPearls Publishing. 2020.

33. Vanderpump MP, Tunbridge WM, French JM, Appleton D, Bates D, Clark F, et al. The incidence of thyroid disorders in the community a twenty-year follow-up of the Whickham Survey. Clin Endocrinol. 1995; 43: 55-68.

34. National Center for Disease Control and Public Health. Subclinical Iodine-deficiency Hypothiroidism and Other forms of Hypothiroidism. 2018.

35. Somwaru LL, Rariy CM, Arnold AM, Cappola AR. The natural history of subclinical hypothyroidism in the elderly the cardiovascular health study. J Clin Endocrinol Metab. 2012; 97: 1962-1969.

36. Mammen JS, McGready J, Oxman R, Chia CW, Ladenson PW, Simonsick EM. Thyroid Hormone Therapy and Risk of Thyrotoxicosis in Community-Resident Older Adults. Findings from the Baltimore Longitudinal Study of Aging. Thyroid. 2015; 25: 979-986.

37. Kong WM, Sheikh MH, Lumb PJ, Naoumova RP, Freedman DB, Crook M, et al. A 6-month randomised trial of thyroxine treatment in women with mild subclinical hypothyroidism. Am J Med. 2002; 113: 442.

38. Magri F, Chiovato L, Croce L, Rotondi M. Thyroid hormone therapy for subclinical hypothyroidism. Endocrine. 2019; 66: 27-34.

39. Pietruszka B Brzozowska A. Homocysteine Serum Level in Relation to Intake of Folate, Vitamins B12, B1 , B2 , and B6 and MTHFR c.665C→T Polymorphism among Young Women. Austin J Nutri Food Sci. 2014; 2: 1052.

40. Ilhan N, Kucuksu M, Kaman D, Ilhan N, Ozbay Y. The 677 C/T MTHFR polymorphism is associated with essential hypertension, coronary artery disease, and higher homocysteine levels. Arch Med Res. 2008; 39: 125-130.

41. Kvaratskhelia T, Kvintradze M, Zarandia M, Kvaratskhelia E, Abzianidze E. Abstracts from the 52nd European Society of Human Genetics (ESHG) Conference Eur J Hum Genet. 2019; 27: 1043-1173.

42. McCully KS. Chemical pathology of homocysteine IV. Excitotoxicity, oxidative stress, endothelial dysfunction, and inflammation. Ann Clin Lab Sci. 2009; 39: 219-232.

43. Kumar A, Palfrey HA, Pathak R, Kadowitz PJ, Gettys TW, Murthy SN. The metabolism and significance of homocysteine in nutrition and health. Nutr Metab . 2017; 14: 78.

44. Brustolin S, Giugliani R. Félix TM Genetics of homocysteine metabolism and associated disorders. Braz J Med Biol Res. 2010; 43: 1-7.