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TRPV2 POLYMORPHISMS CHANGE THE RISK OF TYPE 2 DIABETES - HASHIMOTO THYROIDITIS COMORBIDITY

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Abstract

Context. Thyroid disorders are common in diabetics and related to severe diabetic complications. TRPV2 ion channels have crucial functions in insulin secretion and glucose metabolism which have an important role in the pathophysiology of diabetes. Also, they have a significant effect on various immunological events that are involved in the HT pathophysiology.

Objective. This study aimed to investigate rs14039 and rs4792742 polymorphisms of the TRPV2 ion channels in type 2 diabetes mellitus (T2DM, n=100) Hashimoto thyroiditis (HT, n=70) and comorbid T2DM and HT (T2DM+HT, n=100) patients and control (n=100).

Design. Case-control study

Subject and Methods. RT-PCR genotyping was used to determine rs14039 and rs4792742 polymorphisms with DNA samples of subjects and appropriate primer and probes. Besides, required biochemical analyses were performed.

Results. It was determined that the frequencies of the rs14039 GG homozygote polymorphic genotype and the G allele were significantly higher in T2DM+HT patients compared to the control (p=0.03 and p=0.01, respectively) and that especially the GG genotype increases the risk of T2DM+HT 3.046-fold (p=0.01, OR=3.046). It was detected that the GG genotype increased the risk of HT 2.54-fold (p=0.05, OR=2.541). TRPV2 rs4792742 polymorphisms reduce the risk of HT and T2DM+HT comorbidity almost by half and have a protective effect against HT and T2DM+HT.

Conclusion. The rs14039 GG genotype of the TRPV2 gene significantly increases the risks of development of T2DM+HT and HT disorders, may have a significant role in the pathophysiology of these diseases, also leading to predisposition for their development. Conversely, rs4792742 polymorphic genotypes have a strong protective effect against the HT and T2DM+HT comorbidity.

Key words: TRPV2, Type 2 Diabetes Mellitus, Hashimoto Thyroiditis, Polymorphisms.

INTRODUCTION

Thyroid diseases and Type 2 Diabetes Mellitus (T2DM) are the most commonly observed endocrine diseases in the clinical practice (1-3). Diabetes Mellitus is a disorder emerging due to absolute or functional insulin insufficiency, and leading to disturbances in the lipid, protein, and especially carbohydrate metabolisms (4). Diabetes is a global health problem. According to the data obtained from the International Diabetes Federation, 415 million adults had diabetes in 2015, and this number is predicted to rise to 642 million in 2040. T2DM is the most common type of diabetes, which develops due to reduced insulin sensitivity in the tissues together with developing resistance (3). Thyroid diseases are commonly observed in diabetic patients and are associated with T2DM particularly in older patients (1, 5). A study determined the prevalence of hypothyroidism among T2DM patients to be 13%, statistically significant (1). A meta-analysis revealed that the incidence of subclinical hypothyroidism is higher in T2DM patients compared to healthy subjects. Also, it was suggested that subclinical hypothyroidism might be associated with increased incidence of diabetic complications and that thyroid function screening might be required in T2DM patients (6). Several studies have shown that T2DM patients have a higher prevalence of thyroid diseases compared to non-diabetic adults. These studies have determined different occurrence rates of thyroid diseases among T2DM patients such as 8.6% for subclinical hypothyroidism, 10% for autoimmune thyroiditis, 20.1-22.5% for hypothyroidism (7-10). The most commonly observed autoimmune disease is Hashimoto thyroiditis (autoimmune thyroiditis) today (2). Hashimoto thyroiditis (HT) is an autoimmune disorder characterized by the development of autoantibodies

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against thyroid peroxidase (TPO), thyroglobulin (TG) and thyroid stimulating hormone receptor (TSHr) autoantigens, thus causing hypothyroidism (2,11). For this reason, it was determined that the thyroid gland is infiltrated by plasma cells and lymphocytes and that fibrosis, atrophy and eosinophilic degeneration develop in the parenchyma (12). This disease is more frequent in females when compared to males (11,13). Various genetic and environmental factors are influential in the development of T2DM and HT, which are multifactorial complex polygenic diseases. Many epidemiological family and twin studies have shown that T2DM and HT diseases are strongly hereditary (14, 15). The concordance of T2DM is 70% among monozygotic twins (MZ), whereas it is 20-30% among dizygotic twins. For the individuals who have T2DM in single parent, the risk of developing the disease is 40%, whereas it is 70% for individuals whose both parents have T2DM (16). In MZ, the concordance of autoimmune disease is higher than in dizygotic twins (DZ), reported to be 50% among MZ twins (17). The genome association studies and linkage analyses conducted in the recent years have helped to associate many genomic regions, genes, mutations, and polymorphisms with T2DM and HT (14, 15). It was also asserted that the transient receptor potential cation channel, subfamily V, member 2 (TRPV2) ion channel has important functional roles in insulin secretion that takes part in T2DM pathophysiology, and in the immune system that takes part in the pathophysiology of HT, which is an autoimmune disease (18-20). The TRPV2 ion channels are mostly expressed in neurons, the beta and ductal cells of the pancreas, the spleen, the immune system cells such as mast cells, lymphocytes, and macrophages, together with the gastrointestinal and neuroendocrine cells (18, 21). Several important studies have shown that the TRPV2 ion channels have important functional roles in insulin secretion and the immune system (18, 20, 22-24). This study aimed to evaluate the rs14039 and rs4792742 polymorphisms of the TRPV2 gene, which encodes the TRPV2 ion channel, in patients with T2DM, HT or T2DM + HT. Regarding the known functions of the TRPV2 ion channels, it was suggested that the rs14039 polymorphism of the TRPV2 gene might lead to genetic predisposition in the etiologies of HT and T2DM diseases.

MATERIALS AND METHODS

Ethical Approval

This study was conducted after the required permission was obtained from the Clinical Research Ethical Committee of Kırıkkale University Medical Faculty (2013/14). The participants were informed about the study before participation and written informed consent forms were obtained from all the subjects.

Subjects

The study was conducted with four groups of subjects as follows: T2DM (n = 100), HT (n = 70), T2DM + HT (n = 100) and Control (n = 100). The first 3 groups did not include patients who had diseases/ health problems other than T2DM and HT. The control group consisted of healthy volunteers. For the study, 3 cc of peripheral blood samples were withdrawn into EDTA tubes from the diagnosed patients admitted to the Endocrinology Clinic of the Elazig Training and Research Hospital and the Internal Diseases Clinic of the private Hayat Hospital. For each patient, the detailed medical history, gender, age, laboratory findings (BMI, HbA1c level, fasting blood glucose, TSH level, thyroid USG, anti-TPO and the anti-TG level, etc.) were recorded. The T2DM patients were diagnosed according to the diagnostic criteria established by the American Diabetes Association. These criteria are as follows: (1) blood glucose level ≥126 mg/dL (7.0 mmol/L) after at least 8 hours of fasting, (2) the 2-h plasma glucose level ≥200 mg/dL (11.1 mmol/L) in the 2-h oral glucose tolerance test (OGTT), and (3) the HbA1C ≥ 6.5% (48 mmol/L) (25). The HT patients, considering their complaints, were diagnosed using the presence of TPO and TG antibodies in the blood, the presence of hypothyroidism symptoms, disrupted follicular structure, and reduced echogenicity due to lymphocytic infiltration determined by thyroid USG. The reference values used for TSH were 0.4-4.0 mU/L. When the serum anti-TPO values were less than 40 IU/mL, and the anti-TG levels were less than 35 IU/ mL, the individuals were considered as negative for the disease.

Genotyping

The retrieved blood samples were kept at -20˚C until the time of DNA purification. After being shaken a few times for homogenization, the blood samples were studied in groups of 24, and their DNAs were isolated according to the DNA isolation protocol (Invitrogen; Waltham, MA USA). The primers and probes appropriate for rs14039 and rs4792742 polymorphism sites of TRPV2 (Applied 4351380) were designed by the Applied Biosystems company. A 10 µL reaction mixture was prepared for each sample using the isolated DNA samples, genotyping master

mix, genotyping assay, and nuclease-free water. The prepared mixtures were placed in plate wells using micropipettes, and the plates were covered using plate covers. The plates were placed in the RT-PCR machine (Applied Biosystems® 7500 fast RT-PCR, Waltham, MA USA) and the RT-PCR reaction was realized in a total of 40 cycles, using the temperature and time conditions of 30 seconds at 60˚C, 10 minutes at 95˚C, 15 seconds at 95˚C, 1 minute at 60˚C, and 30 seconds at 60˚C.

Statistical analysis

SPSS (Statistical Package for Social Sciences) for Windows 20.0 (SPSS, Inc., Chicago IL, USA) was used for statistical analysis. The descriptive statistics (mean, standard deviation, frequency, %frequency) were retrieved. Chi-square test was performed to assess Hardy–Weinberg equilibrium. The distribution of the genotype and the allele frequencies of the study and the control groups were conducted with the Chi-square test. The 95%-confidence intervals (95% CIs) and Odds ratios (OR) were also assessed. $p<0.05$ was considered to represent a statistically significant difference.

RESULTS

In the present study, the TRPV2 rs14039 and rs4792742 gene polymorphisms were analyzed in T2DM, HT, T2DM+HT and control groups. The control group consisted of 61 female and 39 male individuals. The mean age of the control group was control age 40.22±14.2 years. The clinical features and demographic characteristics of the patient groups (mean ± SD) are summarized in Table 1. All patient and control groups were found to be in Hardy-Weinberg equilibrium regarding these polymorphisms (Table 2 and 3). The distribution of genotype frequencies of TRPV2 rs14039 and rs4792742 gene polymorphisms among the patient and control groups is shown in Tables 2 and 3. The distribution of allele frequencies in the patient and control groups are shown in Table 4.

Analysis results for the rs14039

Table 1. Clinical features and demographic characteristics of the patients and control (means ±SD)

	T2DM n=100	**T2DM+HT n=100**	**HT n= 70**	**Control n=100**
Gender				
Female, n (%)	62 (62 %)	98 (98 %)	67 (95.7 %)	61 (61%)
Male, n (%)	38 (38 %)	2 (2 %)	3 (4.3 %)	39 (39%)
Age (years)	56 ±10	58 ±7.2	43 ±8	40.22±14.2
Duration of disease (years)	8.3±7.86	DM duration: 6.8 ±6.1 HT duration: 4.2 ±3.97	3.4 ± 3.13	
BMI (kg/m^2)	26.5 ±3.56	30.4 ±5.2	27 ±4.82	24.39±1.9
Fasting glucose (mg/dL)	179.2 ±26.1	161.9 ±35.3	98.8±5.4	95.2±8.4
HA1C (%)	7.96 ±3.01	7.5±2.01	5.2±0.5	4.9±0.28
TSH (mIU/L)	1.84 ± 1.1	2.88±3.03	4.9 ±4.12	1.7 ± 1.43
Anti-TG (IU/mL)	Negative	196.9±134.2	287 ±146	Negative
Anti-TPO (IU/mL)	Negative	357.03±173	289.26 ±197.3	Negative

Table 2. Distribution of genotype frequencies of TRPV2 rs14039 gene polymorphism in the patients and control groups

	TRPV2 rs14039				
	CC	**CG**	**GG**	**H-W eq (p)**	**p (global)**
T2DM (n=100)	46 (46%)	44 (44%)	10 (10%)	0.9121	0.9
p	R	0.65	0.89		
OR (95% CI)	1	1.143 (0.636 to 2.053)	1.065 (0.406 to 2.794)		
HT (n=70)	27 (38.6%)	29 (41.4%)	14 (20%)	0.2353	0.14
p	R	0.46	**0.05**		
OR (95% CI)	1	1.284 (0.658 to 2.505)	2.541 (0.995 to 6.489)		
T2DM+HT (n=100)	37 (37 %)	40 (40 %)	23 (23 %)	0.065	**0.03**
p	R	0.41	**0.01**		
OR (95% CI)	1	1.292 (0.702 to 2.378)	3.046 (0.294 to 7.172)		
Control (n=100)	49 (49%)	41 (41%)	10 (10%)	0.742	

CC: homozygous wild type genotype; CG: heterozygote polymorphic genotype; GG: homozygous polymorphic genotype; n: number; p: p value; H-W eq: Hardy-Weinberg equilibrium; CI: confidence interval; OR: Odds ratio; R: Reference Group.

Polymorphism

The rs14039 genotyping analyses revealed that the frequencies of the rs14039 GG genotype and G allele in patients with T2DM+HT were significantly higher compared to the control group (p=0.03 and p=0.01 respectively, Tables 2 and 4). It was detected that the GG genotype increased the risk of T2DM+HT comorbidity 3.046-fold (p = 0.01 and OR: 3.046), using the CC genotype as a reference. The CG genotype was also found to increase the risk of T2DM+HT comorbidity 1.29-fold; however, this result was found to be statistically insignificant (p = 0.41 and OR: 1.29, Table 2).

There was no significant difference between the HT and Control groups regarding the frequency of rs14039 polymorphic genotype (p = 0.14). As a result of the analysis taking the hereditary CC genotype as a reference, no statistically significant difference was found between the CG polymorphic genotype and the CC genotype (p = 0.46). However, there was an almost significant difference between the GG polymorphic genotype and the CC genotype (p = 0.05), and it was determined that the risk of developing HT was 2.54-fold higher in the GG homozygotic polymorphic genotype compared to the CC genotype (OR = 2.541, Table 2). The frequency of the G polymorphic allele was found to be lower in the HT group when compared to the control group (p= 0.052, Table 4).

There was no significant difference between the T2DM and Control groups regarding the polymorphic genotype and allele frequencies (p = 0.90 and p = 0.74, respectively). As a result of the analysis taking the hereditary CC genotype as a reference, no significant differences were determined between the CG and GG polymorphic genotypes and the CC genotype (p = 0.65 and p = 0.89, respectively, Tables 2 and 4).

Analysis results for the rs4792742 Polymorphism

There were no significant differences between the T2DM + HT and Control groups regarding the frequencies of the rs4792742 polymorphic genotype and the allele (p = 0.49 and p = 0.34, respectively). As the result of the analysis taking the hereditary CC genotype as a reference, no significant differences were determined between the CT and TT polymorphic genotypes and the CC genotype (p = 0.27 and p = 0.27, respectively). In addition, it was determined that the CT (OR = 0.621) and TT (OR = 0.621) were determined to be less effective on the disease, when compared to

Table 3. Distribution of genotype frequencies of TRPV2 rs4792742 gene polymorphism in the patients and control groups

	TRPV2 rs4792742				
	CC	**CT**	**TT**	**H-W eq p value**	**p (global)**
T2DM (n=100)	11 (11%)	45 (45%)	44 (44%)	0.9204	0.97
p	R	0.81	0.85		
OR (95% CI)	1	1.116 (0.446 to 2.793)	1.091 (0.435 to 2.734)		
HT (n=70)	12 (17%)	27 (38.6%)	31 (44.3%)	0.1618	0.58
p	R	0.30	0.45		
OR (95% CI)	1	0.614 (0.241 to 1.559)	0.705 (0.280 to 1.773)		
T2DM+HT (n=100)	18 (18%)	41(41%)	41(41%)	0.179	0.49
p	R	0.27	0.27		
OR (95% CI)	1	0.621 (0.267 to 1.144)	0.621 (0.267 to 1.144)		
Control (n=100)	12 (12%)	44 (44%)	44 (44%)	0.65	

CC: homozygous wild type genotype; CT: heterozygote polymorphic genotype; TT: homozygous polymorphic genotype; n: number; p: p value. H-W eq: Hardy-Weinberg equilibrium; CI: confidence interval; OR: Odds ratio; R: Reference Group.

Table 4. Distribution of allele frequencies of TRPV2 gene rs14039 and rs4792742 polymorphisms in the patients and control groups

	Allele	**T2DM**	**HT**	**T2DM+HT**	**Control**
rs14039	**C**	136 (68%)	83 (59.2%)	114 (57%)	139(69.5%)
	G	64 (32%)	57 (40.7%)	86 (43%)	61 (30.5%)
	p value	0.74	0.05	**0.01**	
	OR	1.072 (0.702 to 1.637)	1.565 (0.996 to 2.459)	1.719 (1.140 to 2.593)	
rs4792742	**C**	67 (33.5%)	51 (36.4%)	77 (38.5%)	68 (34%)
	T	133 (66.5%)	89 (63.5%)	123 (61.5%)	132 (66%)
	p value	0. 91	0.64	0.34	
	OR	1.023 (0.676 to 1.548)	0.899 (0.572 to 1.412)	0.823 (0.547 to 1.238)	

C: C allele; T: T allele; G: G allele; CI: confidence interval; OR (95% CI): Odds ratio; n: number; p: p value.

the hereditary CC genotype (OR = 1, Tables 3 and 4). This suggests that the polymorphic genotypes reduce the risk of HT and T2DM + HT comorbidity almost by half and have a protective effect against HT and T2DM+HT.

No significant differences regarding the polymorphic genotype and allele frequencies were found between the HT and Control groups (p = 0.58 and p = 0.64, respectively). As the result of the analysis taking the hereditary CC genotype as a reference, no significant differences were determined between the CT and TT polymorphic genotypes and the CC genotype (p = 0.3 and p = 0.45, respectively, Tables 3 and 4). Like the T2DM + HT group, the polymorphic genotypes reduced the risk of acquiring HT disease almost by half and had a protective effect against HT (ORCT = 0.614 and ORTT = 0.70). However, this protective effect was not found to be present in the T2DM group (ORCT = 1.16 and ORTT = 1.09, Table 3). There were no significant differences between the T2DM and Control groups regarding the frequencies of genotypes and alleles (p = 0.97 and 0.91, respectively). As the result of the analysis taking the CC genotype as a reference, no statistically significant difference was found between the CT and TT polymorphic genotypes (p>0.05, Tables 3 and 4).

Additionally, it was determined that susceptibility to disease was gender-dependent in the T2DM + HT and HT groups [OR: 31,328 (7,301 to 134,426) p=0,000, OR: 14.279 (4.197 to 48.581) p=0,000, respectively], but not in the T2DM group (p = 0.8). As the result of the analysis using the male gender as the reference category, it was indicated that the probability of developing the disease was 31.328 higher among females in the T2DM + HT group, and 14.27 times higher among females for the HT group.

DISCUSSION

The TRPV2 ion channels have a unique function in the pancreatic β-cells. Under unstimulated conditions, these channels are in the cytoplasm, especially within the endoplasmic reticulum (ER) (20). Following the stimulation of the cells with IGF-1, the TRPV2 channels are translocated to the plasma membrane, and thus, through TRPV2, IGF-1 increases the calcium entry into the cell (by regulating the channel traffic) (26). The translocated TRPV channels increase the entry of sodium ions to the β-cells, as they are permeable to both calcium and sodium. The increased sodium concentration leads to the depolarization of the plasma membrane, and the subsequent voltage-dependent calcium channel activation. These voltage-gated calcium channels contribute to the stimulation-activation coupling and lead to secretion of insulin (18, 26, 27). In addition, it is reported that TRPV2 is a signal molecule that takes part in the insulin action, and that TRPV2 is autocrine-regulated by insulin because the TRPV2 channels function as insulin-dependent regulators of calcium entry. Insulin is released through depolarization of β-cells, the TRPV2 channels that provide extra calcium entry to the cell accumulate on the plasma membrane, and thus increase insulin release. It is indicated that TRPV2 inhibition reduces insulin secretion response and DNA synthesis in β-cells (18). Also, studies have shown that the TRPV2 ion channels in the pancreas serve as a key regulator of the insulin secretion positive-feedback mechanism and glucose homeostasis (21, 28). In this study that we conducted regarding the important roles of TRPV2 ion channels in insulin secretion, we determined that the rs14039 and rs4792742 gene polymorphisms of TRPV2 did not contribute to the predisposition to the disease in patients with T2DM. In addition, several studies have shown that TRPV2 is not only associated with the glucose metabolism, but also with autoimmune diseases (29). It is indicated that TRPV2 ion channels are synthesized and have various roles in cellular functions in the natural and acquired immune system cells (macrophages, mast cells, natural killer cells, monocytes, T- and B-lymphocytes) (19, 28, 30-32). The non-selective cation channel TRPV2 has been suggested to regulate the receptor aggregation and substrate binding in macrophages. It was determined that in mice with TRPV2 knock-out, bacterial load and mortality was higher in the Listeria monocytogenes infection. The findings of this study showed the fundamental importance of TRPV2 in natural immunity and the early stages of macrophage phagocytosis (31). Another study suggested that the activation of TRPV2 provide the inflow of Ca^{2+} ions, and the increase in Ca^{2+} concentrations induce the degranulation of mast cells (32). These studies emphasize the possible important functions of TRPV2 channels in multifarious cell types of the immune system (19, 28). Hashimoto thyroiditis, one of the diseases handled in this study, is an autoimmune disorder with an immune pathophysiology (33), and thus was hypothesized to be related to the rs14039 polymorphism of the TRPV2 ion channel.

A study has determined that the c.-323A>G single nucleotide gene polymorphism of the TRPV2 gene took part in causing oversensitivity to osmotic stimulants in the asthma, which also has an immune

pathophysiology (34). Another study conducted among children with asthma found a strong correlation between asthma and the TRPV2 rs14039 gene polymorphism (35). It was also reported that several TRPV2 gene polymorphisms affected sensitivity to fibromyalgia (36). Even though various TRPV2 gene polymorphisms were studied in the context of several diseases such as asthma and fibromyalgia, there were no studies that examined the rs14039 and rs4792742 gene polymorphisms of TRPV2 for T2DM, HT, and T2DM + HT comorbidity. For this reason, this study is the first, and it was determined that the rs14039 GG genotype leads to 3.046-fold increased disease risk in T2DM + HT patients and 2.5-fold raised disease risk in HT patients. Surprisingly, in this study, it was determined that, although not related to T2DM, the TRPV2 rs14039 GG genotype was found to be related to the HT and T2DM + HT patient groups. Thus, it can be suggested that the TRPV2 rs14039 GG genotype may be associated with the development of HT disease, and T2DM + HT comorbidity and acting as an important factor in the pathophysiology of these diseases. It was also determined that the TRVP2 rs4792742 polymorphic genotypes reduced the development risk of HT and T2DM + HT almost by half, thus having a protective effect against HT and T2DM + HT.

Thyroid hormones act on glucose metabolism (37,38) and the comorbidity of T2DM and HT lead to an increase in the incidence of several diseases (39-41). Hashimoto thyroiditis is a disease that generally progresses with hypothyroidism (2) and studies have determined that the risks of nephropathy, retinopathy, and cardiovascular diseases are significantly increased in T2DM patients with subclinical hypothyroidism, compared to euthyroid T2DM patients (39-41). Similarly, it is indicated that hypothyroidism can affect the development of diabetic complications such as nephropathy, retinopathy and arterial diseases among diabetic patients (6,10,39).

In conclusion, as stated before, the comorbidity of T2DM and HT diseases increases the prevalence of several diseases, and thyroid hormonal disorders enhance the diabetic complications in patients with T2DM. Therefore, this study has scientific importance regarding the physiological functions of the TRPV2 rs14039 GG homozygotic polymorphic genotype, which has been determined to increase the risk of HT and T2DM + HT comorbidity, and further studies are required using larger samples.

Conflict of interest

The authors declare that they have no conflict of interest.

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