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Original Article

Frequency of UCP2 45-bp Ins/Del polymorphism in Saudi population from Jazan area and its association with autoimmune hypothyroidism *UCP2* 45-bp Ins/Del frequency in hypothyroidism

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ABSTRACT

Objectives: Autoimmune hypothyroidism (AHT) is a common endocrine disorder. Although the exact cause of AHT is not yet understood, genetic factors may play a major role. Uncoupling protein 2 (UCP2) is a member of mitochondrial protein family involved in the regulation of cellular metabolism. An important functional polymorphism in the *UCP2* gene, 45-bp insertion/deletion (ins/del) polymorphism, has been linked to certain clinical conditions. However, an association between the 45-bp ins/del polymorphism and AHT has not yet been established.

Methods: In this study, about 259 blood samples were collected from, patients with AHT and age-matched healthy control subjects. DNA was extracted for *UCP2* 45-bp ins/del polymorphisms genotyping, using a standard polymerase chain reaction technique. The distribution of different genotypes was determined in both groups and possible association with AHT was also assessed by logistic regression analysis using the Del/Del variant as a reference genotype.

Results: The frequency of the *UCP2* 45-bp ins/del polymorphism in the total study population was 49.04%, 40.15%, and 10.81% for Del/Del, Ins/Del, and Ins/Ins genotypes, respectively. The logistic regression analysis showed crude odds ratios (ORs), respectively, with their 95% confidence intervals (CIs) and *P*-values in codominant (Del/Ins) (OR = 1.53, CI = 0.89–2.60, *P* = 0.17), codominant (Ins/Ins) (OR = 0.75, CI = 0.34–1.74, *P* = 0.53), dominant (OR = 1.30, CI = 0.79–2.16, *P* = 0.37), and recessive (OR = 0.62, CI = 0.29–1.36, *P* = 0.30) inheritance models tested, where none of which were statistically significant.

Conclusion: Our data revealed the distribution of the *UCP2* 45-bp ins/del polymorphisms in Jazan area and confirmed the lack of association between these genetic variants and the development of AHT.

Keywords: Autoimmune hypothyroidism, Saudi Arabia, uncoupling protein 2

Introduction

Autoimmune thyroid diseases (AITDs) are among the most common endocrine disorders. These diseases are highly prevalent worldwide, affecting about 5% of the population.[1,2] Autoimmune hypothyroidism (AHT), most commonly, Hashimoto's thyroiditis (HT), is a tissue-destructive disease characterized by the presence of anti-thyroid antibodies and T-lymphocyte infiltration of the thyroid gland, reducing its capacity to produce thyroid hormones (hypothyroidism). Although the exact cause triggering AHT is not yet known, genetic factors may play a major role.[3]

Uncoupling protein 2 (UCP2) belongs to a mitochondrial protein family that also includes UCP1 and UCP3. These UCPs act as proton carriers in the mitochondria. They work to increase proton influx through the inner mitochondrial membrane without ATP synthesis, resulting in efficient caloric consumption and heat generation.[4] UCPs are also involved in the limitation of cellular free radicals and reactive oxygen species (ROS).[5] Among these proteins, UCP2 is the most ubiquitously expressed, with greater impact on mitochondrial function and is, therefore, the most frequently studied. UCP2 is encoded by the *UCP2* gene located in the q arm of chromosome 11, spanning 8 kb with 8 exons, and a GC rich

promoter region.[6] The *UCP2* 45-bp ins/del polymorphism is a common polymorphism resulting from removal or retention of a 45-bp DNA sequence in the 3' untranslated region of exon 8 of *UCP2*.[7] This polymorphism does not influence the level of *UCP2* mRNA expression; however, it can affect mRNA stability.[7,8] Therefore, it might have a significant influence on energy metabolism. Few studies have reported an association between the 45-bp ins/del polymorphism and obesity or type 2 diabetes,[9,10] while most studies found no association with these conditions.[11,12]

A link between AHT and UCP2 mitochondrial protein has also not yet been established. However, several studies have suggested a possible role for this protein in the regulation of human immune function. For example, UCP2 is expressed in a number of immune cells such as T lymphocytes and natural killer cells.[13] The *UCP2* knock-out mice (*Ucp2*$^{-/-}$) were shown to have more resistance to *Toxoplasma gondii* infection as a result of increased ROS generation inside macrophages compared to wild-type mice (*Ucp2*$^{+/+}$).[14] Moreover, elevated ROS level due to lack of UCP2 has also been linked to high low-density lipoprotein cholesterol levels, the risk of developing inflammatory atherosclerotic plaques,[15] and autoimmune diabetes.[16] In other studies, UCP2 overexpression in monocytes was associated with inhibit monocyte activation, adhesion, and trans-endothelial migration.[17,18] Recently, UCP2 was shown to affect immune cells through activation of the T-cell mitogen-activated protein kinase pathway, stimulation of cytokines, and nitric oxide production.[19] Based on these functions of the UCP2 in the immune response, we hypothesized that polymorphisms in the *UCP2* gene might disrupt the function of the protein and contribute to immune diseases.

Regardless of the large number of association studies in the past decades investigating the role of the UCP2 45bp ins/del polymorphism in the development of human disease, the association with AHT has not yet been established. Therefore, the current study aimed to test any association between these polymorphisms and AHT in samples from Jazan Province, southern west of Saudi Arabia. This will contribute to better understanding of the genetic architecture of the AHT disease.

Methods

Subjects

In the current case–control study, a total of 259 age-matched (30–60 years) male and female subjects were recruited from King Fahad Central Hospital, the outpatient clinic (control) and the Endocrine and Diabetes Center (hypothyroid patients) in the Jazan area, southern west of Saudi Arabia. Samples were collected during the period from November 2018 to March 2019. Inclusion criteria for AHT patients were high levels of thyroid-stimulating hormone and low levels of free thyroxine at the time of diagnosis, as well as testing positive for anti-thyroid peroxidase and/or anti-thyroglobulin autoantibodies. Subjects in the healthy control group were included if they had no previous history of thyroid or other autoimmune disease, severe illness, or a chronic inflammatory condition. The study was approved by the Jazan Research Ethics Committee of the General Directorate of Health Affairs (Jazan), Ministry of Health, Saudi Arabia. Written informed consent was obtained from all participants.

Baseline measurements

A standard questionnaire was used to collect basic anthropometric and clinical data from all participants. Information regarding the subjects' gender, age, height, weight, heart rate, blood pressure, and family history of AITD was directly obtained during an interview at the time of sample collection. The body mass index (BMI) was calculated according to the formula: BMI = weight (kg)/height (m) squared. The presence of goiter was assessed by direct inspection and palpation during physical examination; it was considered absent if no palpable or visible thyroid enlargement was observed.

Genotyping

Peripheral blood samples were collected in 5 mL ethylenediaminetetraacetic acid-containing tubes from all participants and subjected to genomic DNA extraction using a standard protocol.[20] The extracted DNA samples were then used for subsequent genotyping of the *UCP2* 45-bp ins/del polymorphism using standard polymerase chain reaction (PCR) techniques. Briefly, 1 μL of extracted template DNA and 1 μL (0.1 μg each) of forward (5' CAG TGA GGG AAG TGG GAG G 3') and reverse (5' GGG GCA GGA CGA AGA TTC 3') primers were mixed and the volume made up to 12.5 μL with sterile water. The mixture was then added to an equal volume (12.5 μL) of the 2X PCR master mix (AccuPower PCR PreMix, BIONEER, Daejeon, Korea) to reach a total volume of 25 μL. The following thermal profile was used for the PCR amplification: Initial denaturation at 95°C for 4 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 58°C for 30 s, and elongation at 72°C for 30 s, followed by a final extension step at 72°C for 10 min. Samples were then run on 1% agarose gel and stained with SYBER safe DNA gel stain. The deletion (Del) and insertion (Ins) alleles were then distinguished by the appearance of 412-bp and 457-bp bands on the agarose gel, respectively [Figure 1].

The figure shows a representative example of an agarose gel of the *UCP2* 45-bp ins/del polymorphism PCR genotyping results. The homozygous Del/Del genotype is represented by a single DNA band of 457 bp while the heterozygous genotype Ins/Del is represented by two bands at 457 and 412 bp, and the homozygous insertion genotype Ins/Ins is represented by one single band at 457 bp.

Statistical analysis

Data are presented as mean ± standard deviation or percentages for non-continuous variables. Statistical significance of data was assessed by a simple student *t*-test or Fisher's exact test where both variables were dichotomous. Chi-square (χ^2) analysis was used to test for Hardy–Weinberg equilibrium (HWE). To evaluate the association between the presence of the *UCP2* ins/del polymorphism and the likelihood of developing AHT, logistic regression analysis was performed using the Del/Del genotype as a reference non-exposed genotype. The crude odds ratios (ORs) with their 95% confidence intervals (CIs) and *P*-values based on Fisher's exact test were used to indicate statistical significance. The analyses were conducted at multiple models of inheritance: A codominant model (Del/Del vs. Ins/Del or Ins/Ins), a dominant model (Del/Del vs. X/Ins), and a recessive model (Del/X vs. In/Ins). Statistical analyses were performed using GraphPad Prism 8 software (California, USA). Statistical significance was reported when $P < 0.05$ was obtained.

Results

Baseline characteristics of the study population

The baseline characteristics of the control individuals and patients with AHT are listed in Table 1. The data showed a significant difference in gender distribution between the two groups ($P < 0.0001$) and a statistically significant reduction in the mean height of the subjects in the AHT group (1.57 ± 0.08) compared to the control group (1.64 ± 0.09) with $P < 0.0001$. The average age of the control group was 37.1 ± 6.6 years and of the AHT group was 39.6 ± 12.16 years, and no statistical difference was found between the groups ($P = 0.144$). AHT patients demonstrated a higher mean BMI (30.56 ± 6.13), lower systolic blood pressure (104.0 ± 10.5), and lower heart rate (68.0 ± 8.9) compared to the control subjects (24.81 ± 5.9, 111.0 ± 8.7, and 79.8 ± 7.1, respectively). In addition, patients with AHT also exhibited a higher occurrence of goiter (70.2%) and increased frequency of AITD in the family history (81.3%) compared to the control (0% and 13.3%, respectively).

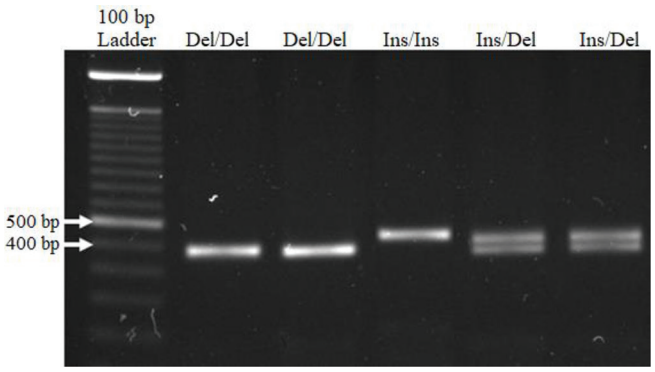

Figure 1: PCR detection of the UCP2 45-bp ins/del polymorphism

Prevalence of the *UCP2* 45-bp ins/del polymorphism in the study population

Figure 2 shows the overall frequency of the *UCP2* 45-bp ins/del polymorphism in healthy individuals and patients with AHT in the study population. The Del/Del genotype was the most frequently observed genotype (49.04%) followed by the Ins/Del genotype (40.15%) and the Ins/Ins genotype (10.81%). In an effort to determine whether our data were in HWE, the observed genotype frequencies were compared to the expected frequencies. The data comparison showed similar values with a Chi-square = 0.92 and $P = 0.34$, indicating that all genotypes have reached the HWE [Table 2].

Association studies

In the codominant model, the distribution of the *UCP2* ins/del polymorphism in patients with AHT was 53.2% for the Del/

Table 1: Baseline characteristics of the control and autoimmune hypothyroid (AHT) subjects

Variables	Control (n=165)	HT (n=94)	*P*
Gender n (Male/Female)	85/80	7/87	<0.0001*
Age (years)	37.1± 6.6	39.6 ± 12.16	0.144
Height (m)	1.64 ± 0.09	1.57 ± 0.08	<0.0001
Weight (kg)	67.07 ± 13.8	72.69 ± 15.17	0.0032
Body mass index (kg/m²)	24.81 ± 5.9	30.56 ± 6.13	<0.0001
Systolic blood pressure (mm Hg)	111.0 ± 8.7	104.0 ± 10.5	0.006
Heart rate (beats/min)	79.8 ± 7.1	68.0 ± 8.9	<0.001
Goiter n (%)	0 (0%)	66 (70.2)	<0.001
Family history of autoimmune thyroid disease n (%)	22 (13.3)	56 (81.3)	<0.0001*

Data are mean ± SD or percentage. * Fischer exact test.

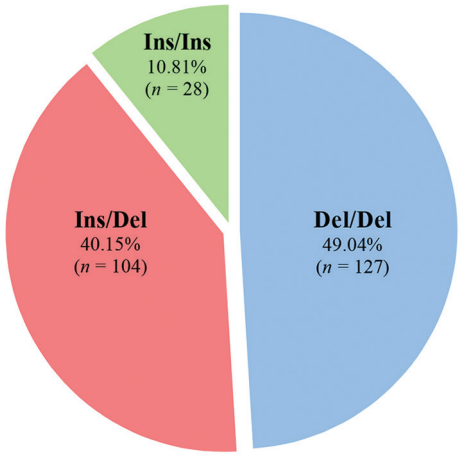

Figure 2: Distribution of the UCP2 45-bp ins/del genetic variants in the study population.
(A pie chart indicating the incidence and relative frequency of different UCP2 ins/del genetic variants; Del/Del, Ins/Del and Ins/Ins genotypes, in the study population [n = 259])

Del genotype, 33.0% for the Del/Ins genotype, and 13.8% for Ins/Ins genotype compared to 46.7%, 44.2%, and 9.1%, respectively, in the healthy control subjects. Using the Del/Del variant as the reference genotype, logistic regression analysis showed crude ORs, respectively, with their 95% CIs and *P*-value in the codominant (Del/Ins) (OR = 1.53, CI = 0.89–2.60, *P* = 0.17), and Ins/Ins (OR = 0.75, CI = 0.34–1.74, *P* = 0.53). In the dominant model, the distribution of the X/Ins genotype (in the cases and control group, respectively) was 46.8% and 90.9% compared to the reference genotype Del/Del; 46.7% and 53.2% (OR = 1.30, CI = 0.79–2.16, *P* = 0.37). In the recessive model, the distribution of the Ins/Ins genotype was; 13.8% and 9.1% compared to the reference genotype Del/X; and 86.2% and 10.8% (OR = 0.62, CI = 0.29–1.36, *P* = 0.30) [Table 3]. Therefore, none of the above logistic regression analysis was statistically significant. Analysis based on allelic frequencies, the Ins allele distribution was 30.3% and 31.2 (in the cases and control group, respectively) compared to the reference allele Del; 69.7% and 68.8% and this gave an OR = 1.04, CI = 0.71–1.53, *P* = 0.84, for the insertion allele to develop AHT, which was also not statistically significant.

Distribution of the UCP2 45-bp ins/del genetic variants in the study population is summarized in [Figure 2].

Discussion

AHT is one of the most common endocrine abnormalities worldwide and the disease prevalence is higher in females than in males.[21] Hashimoto's disease is an autoimmune disease that has been recognized as one of the main causes of hypothyroidism and the exact cause of this disease is still not known.[22] The identification of candidate genes responsible for this disease is, therefore, an important step toward understanding the molecular mechanisms of the disease. Our data initially showed a statistically significant difference in the gender and final heights between the two studied populations. These findings are due to an extremely low number of male patients in the AHT group, as the disease is more common in females than in males. In addition, the AHT group exhibited higher BMI than the control group and this is consistence with the fact that the obesity is more prevalent patients with hypothyroidism.[23] The study also explored the frequency of the UCP2 45-bp Ins/del genetic variants in Jazan region for the 1st time and tested a possible association between this polymorphism and AHT. We found that the Del/Del genotype is the most common genotype in Jazan province (49.04%), followed by the Del/Ins genotype (40.15%), with the Ins/Ins variant being the least frequent genotype (10.81%). Similar findings were also reported in Saudi Arabia[20,24] and nearby countries such as Iran.[25]

Our data also found a lack of association between the *UCP2* 45-bp ins/del polymorphism and the development of AHT in Saudi Arabia. Therefore, this polymorphism might not be a risk factor for the disease. To the best of our knowledge, this is the first study to investigate such a relationship. However, the *UCP2* 45-bp ins/del polymorphism has been linked to other clinical conditions, such as metabolic syndrome and obesity.[20,25-27] Concerning autoimmune disorders, the *UCP2* 45-bp ins/del polymorphism has not been shown to be involved in any disease; however, other polymorphisms in the same gene have been reported to influence both autoimmune and inflammatory conditions. For instant, the -866 G/A polymorphism in the *UCP2* gene has been linked to rheumatoid arthritis and multiple sclerosis.[28] In addition, the A allele was found to have a protective role against chronic inflammatory conditions such as systemic lupus erythematosus.[29]

Table 2: Consistency with Hardy-Weinberg Equilibrium (HWE)

Genotype	Observed	Expected	χ^2 (*P*)*
Del/Del	127	123.7	0.92 (0.34)
Ins/Del	104	110.6	
Ins/Ins	28	24.7	

* χ^2 test *p*-value with 1 degree of freedom, (if p < 0.05 - not consistent with HWE).

Table 3: Genotype and allele distribution of the *UCP2* 45-bp ins/del polymorphism among AHT patients and healthy control subjects

	Total (n=259) n (%)	Control (n=165) n (%)	HT (n=94) n (%)	Odds ratio (95% CI), *P*
Codominant Model				
Del/Del	127 (49.0)	77 (46.7)	50 (53.2)	1.0
Del/Ins	104 (40.1)	73 (44.2)	31 (33.0)	1.53 (0.89 - 2.60), 0.17
Ins/Ins	28 (10.8)	15 (9.1)	13 (13.8)	0.75 (0.34 - 1.74), 0.53
Dominant Model				
Del/Del	127 (49.0)	77 (46.7)	50 (53.2)	1.0
*X/Ins	132 (51.0)	88 (90.9)	44 (46.8)	1.30 (0.79 - 2.16), 0.37
Recessive Model				
Del/X	231 (89.2)	150 (10.8)	81 (86.2)	1.0
Ins/Ins	28 (10.8)	15 (9.1)	13 (13.8)	0.62 (0.29 -1.36), 0.30
Alleles				
Del	358 (69.1)	227 (68.8)	131 (69.7)	1.0
Ins	160 (30.9)	103 (31.2)	57 (30.3)	1.04 (0.71 - 1.53), 0.84

*Where X can be Del or Ins allele.

Conclusion

We report here the distribution of various *UCP2* ins/del polymorphism genotypes in a study population from Jazan province, located in south west of Saudi Arabia. This study did not find any significant link between this polymorphism and AHT. One major limitation of the present study is the relatively small sample size. Therefore, other studies with a larger sample size and different ethnic groups are required to validate our findings.

Ethics Approval and Consent to Participate

Ethical approval was taken from Jazan Research Ethics Committee of the General Directorate of Health Affairs, Ministry of Health, Saudi Arabia. Written informed consent was obtained from all participants.

Availability of Data and Material

The data used in this study are available and will be provided by the corresponding author on a reasonable request.

Conflicts of Interest

Authors declared that they have no conflicts of interest.

Funding Statement

None.

Authors' Contributions

All authors involved in data collection, data interpretation, and manuscript drafting.

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