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

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. 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. This polymorphism does not influence the level of UCP2 mRNA expression; however, it can affect mRNA stability. 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, while most studies found no association with these conditions.

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. 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+/+). Moreover, elevated ROS level due to lack of UCP2 has also been linked to high-density lipoprotein cholesterol levels, the risk of developing inflammatory atherosclerotic plaques, and autoimmune diabetes. In other studies, UCP2 overexpression in monocytes was associated with inhibit monocyte activation, adhesion, and trans-endothelial migration. 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. 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. 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 ($\chi^2$) 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. Ins/Ins). Statistical analyses were performed using GraphPad Prism 8 software (California, USA). Statistical significance was reported when $P < 0.05$ was obtained.

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/

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](image1)

![Figure 2: Distribution of the UCP2 45-bp ins/del genetic variants in the study population.](image2)

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. * Fisher exact test.
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 Ins/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. 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. 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 consistency with the fact that the obesity is more prevalent patients with hypothyroidism. 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 and nearby countries such as Iran.

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. 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 instance, the -866 G/A polymorphism in the UCP2 gene has been linked to rheumatoid arthritis and multiple sclerosis. In addition, the A allele was found to have a protective role against chronic inflammatory conditions such as systemic lupus erythematosus.

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

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

* \( \chi^2 \) 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

| Genotype/Allele | 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.

Acknowledgment

The authors want to thank all volunteer subjects who effectively and kindly participated in this study. This study was supported financially by a grant provided by the Deanship of Scientific Research at Jazan University and the work was conducted as the Medical Research Center at the University.

References

1. McGrogan A, Seaman HE, Wright JW, De Vries CS. The incidence of autoimmune thyroid disease: A systematic review of the literature. Clin Endocrinol (Oxf) 2008;69:687-96.
2. McLeod DS, Cooper DS. The incidence and prevalence of thyroid autoimmunity. Endocrine 2012;42:252-65.
3. Tomer Y. Genetic susceptibility to autoimmune thyroid disease: Past, present, and future. Thyroid 2010;20:715-25.
4. Brand MD, Esteves TC. Physiological functions of the mitochondrial uncoupling proteins UCP2 and UCP3. Cell Metab 2005;2:85-93.
5. Murphy MP, Echtaf KS, Blakie FH, Asin-Cayuela J, Cocheme HM, Green K, et al. Superoxide activates uncoupling proteins by generating carbon-centered radicals and initiating lipid peroxidation: Studies using a mitochondria-targeted spin trap derived from alpha-phenyl-N-tert-butylnitronate. J Biol Chem 2003;278:48534-45.
6. Pecqueur C, Cassard-Douclier AM, Rainbault S, Miroux B, Fleury C, Gelly C, et al. Functional organization of the human uncoupling protein-2 gene, and juxtaposition to the uncoupling protein-3 gene. Biochem Biophys Res Commun 1999;255:40-6.
7. Walder K, Norman RA, Hanson RL, Schrauwen P, Neverova M, Jenkinson CP, et al. Association between uncoupling protein polymorphisms (UCP2-UCP3) and energy metabolism/obesity in Pima Indians. Hum Mol Genet 1998;7:1431-5.
8. Lindholm E, Klannemark M, Agardh E, Groop L, Agardh CD. Putative role of polymorphisms in UCP1-3 genes for diabetic nephropathy. J Diabetes Complications 2004;18:103-7.
9. Dalgaard LT, Pedersen O. Uncoupling proteins: Functional characteristics and role in the pathogenesis of obesity and Type II diabetes. Diabetologia 2001;44:946-65.
10. Jia JJ, Zhang X, Ge CR, Jois M. The polymorphisms of UCP2 and UCP3 genes associated with fat metabolism, obesity and diabetes. Obes Rev 2009;10:519-26.
11. Dalgaard LT, Andersen G, Larsen LH, Sorensen TI, Andersen T, Drivsholm T, et al. Mutational analysis of the UCP2 core promoter and relationships of variants with obesity. Obes Res 2003;11:1420-7.
12. Ochoa MC, Santos JL, Azcona C, Moreno-Aliaga MJ, Martinez-Gonzalez MA, Martinez JA, et al. Association between obesity and insulin resistance with UCP2-UCP3 gene variants in Spanish children and adolescents. Mol Genet Metab 2007;92:351-8.
13. Rupprech A, Brauer AU, Smorodchenko A, Goy J, Hilse KE, Shabalina IG, et al. Quantification of uncoupling protein 2 reveals its main expression in immune cells and selective up-regulation during T-cell proliferation. PLoS One 2012;7:e41406.
14. Arsenijevic D, Onuma H, Pecqueur C, Raimbault S, Manning BS, Miroux B, et al. Disruption of the uncoupling protein-2 gene in mice reveals a role in immunity and reactive oxygen species production. Nat Genet 2000;26:435-9.
15. Blane J, Alves-Guerra MC, Esposito B, Rouset S, Gourdy P, Ricquier D, et al. Protective role of uncoupling protein 2 in atherosclerosis. Circulation 2003;107:388-90.
16. Emre Y, Hurtaud C, Karaca M, Nubel T, Zavala F, Ricquier D. Role of uncoupling protein UCP2 in cell-mediated immunity: How macrophage-mediated insults is accelerated in a model of autoimmune diabetes. Proc Natl Acad Sci U S A 2007;104:19085-90.
17. Ryu JW, Hong KH, Maeng JH, Kim JB, Ko J, Park JY, et al. Overexpression of uncoupling protein 2 in THP1 monocytes inhibits beta2 integrin-mediated firm adhesion and transendothelial migration. Arterioscler Thromb Vasc Biol 2004;24:864-70.
18. Liu DQ, Guo YL, Bian Z, Chen YY, Chen X, Liu Y, et al. Uncoupling protein-2 negatively regulates polymorphonuclear leukocytes chemotaxis via modulating [Ca2+] influx. Arterioscler Thromb Vasc Biol 2010;30:575-81.
19. Emre Y, Nubel T. Uncoupling protein UCP2: When mitochondrial activity meets immunity. FEBS Lett 2010;584:1437-42.
20. Kaabi YA. The deletion polymorphism in exon 8 of uncoupling protein 2 is associated with severe obesity in a Saudi Arabian case-control study. Indian J Endocrinol Metab 2018;22:200-3.
21. Kostoglou-Athanassiou I, Natelles K. Hypothyroidism-new aspects of an old disease. Hippokratia 2010;14:82-7.
22. Zaletel K, Gaberscek S. Hashimoto’s thyroiditis: From genes to the disease. Curr Genomics 2011;12:576-88.
23. Song RH, Wang B, Yao QM, Li Q, Jia X, Zhang JA. The impact of
obesity on thyroid autoimmunity and dysfunction: A systematic review and meta-analysis. Front Immunol 2019;10:2349.
24. Jiffri E. Association of the UCP2 45-bp insertion/deletion polymorphism with diabetes Type 2 and obesity in Saudi population. Egypt J Med Hum Genet 2012;13:257-62.
25. Hashemi M, Rezaei H, Kaykhaei MA, Taheri M. A 45-bp insertion/deletion polymorphism of UCP2 gene is associated with metabolic syndrome. J Diabetes Metab Disord 2014;13:12.
26. Kovacs P, Ma L, Hanson RL, Franks P, Stumvoll M, Bogardus C, et al. Genetic variation in UCP2 (uncoupling protein-2) is associated with energy metabolism in Pima Indians. Diabetologia 2005;48:2292-5.
27. Duarte NL, Colagiuri S, Palu T, Wang XL, Wilcken DE. A 45-bp insertion/deletion polymorphism of uncoupling protein 2 in relation to obesity in Tongans. Obes Res 2003;11:512-7.
28. Vogler S, Goedde R, Miterski B, Gold R, Kroner A, Koczan D, et al. Association of a common polymorphism in the promoter of UCP2 with susceptibility to multiple sclerosis. J Mol Med (Berl) 2005;83:806-11.
29. Yu X, Wieczorek S, Franke A, Yin H, Pierer M, Sina C, et al. Association of UCP2-866 G/A polymorphism with chronic inflammatory diseases. Genes Immun 2009;10:601-5.