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ORIGINAL RESEARCH

The Association of UCP2-866 G/A Genotype with Autoimmune Hypothyroidism in the Southwestern Saudi Arabia Population

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Introduction: Autoimmune hypothyroidism (AHT) is a widespread disease that disproportionately affects women over men. It is characterized by the presence of autoantibodies that lead to the dysfunction of the thyroid gland. The exact cause of this process is unknown; however, some factors, such as genetic factors, may be to blame. *The uncoupling protein 2 (UCP2) gene* encodes uncoupling protein 2, which has been linked to several pathogeneses; however, the link between UCP2-866 G/A polymorphism and AHT has yet to be investigated. Thus, we investigate the potential relationship between UCP2-866 G/A polymorphism and AHT.

Methods: A total of 158 subjects participated in this study, they were either control or AHT patient, and genotyping was performed using a polymerase chain reaction.

Results: The frequencies of UCP2-866 G/G, G/A, and A/A in the control subject were 34%, 51%, and 15%, respectively, whereas these frequencies in the AHT were 43%, 46%, and 10%.

Conclusion: The study concludes a significant relationship between UCP2-866 G/A polymorphism and AHT, with a carrier subject of the –866 A allele being 3 times more likely to suffer from AHT than wild-type carriers in the study population.

Keywords: autoimmune hypothyroidism, AHT, uncoupling protein 2, UCP2-866, Hashimoto's thyroiditis

Introduction

Hypothyroid disease is a common disease worldwide, it affects 5% of the population, and females are affected more than 10 times more than males. The thyroid gland becomes autoimmune for unknown reasons, resulting in Graves' disease and Hashimoto's thyroiditis. This disease is distinguished by the presence of autoantibodies and T-cell infiltration into the gland tissue,[1] and as a result, the hormone production function is compromised.[2,3] The pattern of the disease and the precise cause that triggers the autoimmune response are still unknown, but genetic factors have been revealed to be one of the critical factors that lead to autoimmune hypothyroidism (AHT), as well as other thyroid abnormalities.[4–6]

Uncoupling protein 2 (UCP2) is a protein found in a variety of tissues including the spleen, macrophages, and T-cells. It is a sort of mitochondrial protein family, encoded by the *UCP2* gene, which is located on the q arm of chromosome 11.[7] It acts as antioxidative stress, decoupling substrate oxidation from ATP synthesis, and down-regulates reactive oxygen species (ROS) produced at sites of inflammation and injury.[8,9] The UCP2–866 G/A (rs659366) polymorphism in the promoter region of the UCP2 gene has recently been linked to chronic illness in various ethnicities such as ischemic stroke, obesity, and diabetes.[10–12] Furthermore, other studies indicate a link with chronic inflammatory diseases like systemic lupus erythematosus, Crohn's disease and ulcerative colitis.[13] Although these studies have attempted to determine the associations between UCP2–866 G/A and other diseases, the relationship between AHT and this polymorphism has not been investigated, which is what we aimed for in the current study.

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Materials and Methods

Subject and Blood Samples

The Jazan Research Ethics Committee of the General Directorate of Health Affairs (Jazan), Ministry of Health, Saudi Arabia, approved the current study, which complies with the Declaration of Helsinki. All participants provided written informed consent. A total of 158 age-matched (30–60 years) male and female subjects were recruited for the current case–control study from King Fahad Central Hospital, the outpatient clinic (control), and the Endocrine and Diabetes Center (hypothyroid patients) in the Jazan area of southwest Saudi Arabia. These subjects were chosen at random from a population of Saudis primarily from the Jazan region. Samples were collected during the period from November 2018 to March 2019. At the time of diagnosis, AHT patients had high levels of thyroid-stimulating hormone and low levels of free thyroxine, as well as anti-thyroid peroxidase and/or anti-thyroglobulin autoantibodies. The healthy control group included subjects who had no history of thyroid or other autoimmune diseases, severe illness, or a chronic inflammatory condition.

DNA Extraction and Genotype Determination

The samples were received in ethylenediaminetetraacetate (EDTA) tubes. According to the manufacturer's instructions, DNA was extracted using the GeneJET Whole Blood Genomic DNA Purification Mini Kit (Thermofisher, Paisley, United Kingdom). The purified DNA was assessed for quality and quantity using NanoDrop 200 spectrophotometer (Thermofisher, Paisley, United Kingdom). Using standard polymerase chain reaction (PCR) techniques, a primer was used to capture the single nucleotide variant corresponding to the UCP2−866 G/A polymorphism (rs659366). In brief, 50 ng of DNA template was mixed with 0.5 µM of each of forward (5' CAC GCT GCT TCT GCC AGG AC 3') and reverse (5' AGG CGT CAG GAG ATG GAC CG 3') primers in a volume of 12.5 µL of sterile water. To make a total volume of 25 L, the mixture was mixed with an equal volume (12.5 µL) of the 2X PCR master mix (Phusion Green Host Start II High-Fidelity PCR Master Mix) (Thermo Fisher Scientific, Paisley, UK). The following thermal profile was used for the PCR amplification: Initial denaturation at 95°C for 4 minutes, then 35 cycles of denaturation at 94°C for 30 seconds, annealing at 58°C for 30 seconds, and elongation at 72°C for 30 seconds, followed by a 10-minute final extension step at 72°C. PCR products were digested by *Mlu I* restriction enzyme (NEB, Ipswich, MA, USA) and separated on 2% agarose gel electrophoresis. Due to the lack of a Mlu I site, the (−866)A/A genotype was identified by a single 363 bp fragment, whereas the wild-type (−866)G/G genotype was digested into 295 bp and 68 bp fragments.[12,14]

Statistical Analysis

For continuous variables, the results were presented as mean ± standard deviation, and for discrete variables, as frequencies with percentages. Parametric test Student–t for unequal variances was applied for testing the significant difference in scale variables. The non-parametric test chi-square was used to examine the relationship between the ordinal variables. Hardy–Weinberg Equilibrium consistency was evaluated by using the chi-square test. Bivariate logistic regression analysis was performed using the crude odds ratio with a 95% confidence interval and *P*-value less than 0.05 considered statistically significant to assess the association between the presence of UCP2-866 G/A polymorphism and the likelihood of developing hypothyroidism.

Result

There were 158 people in total (91 control and 67 with hypothyroidism). All hypothyroidism subjects were female; age and height were statistically different between the two groups (Table 1). The distribution of the UCP2-868 G/A genetic variants in the study population was similar, with a chi-square = 0.23 and P = 0.89, indicating that all genotypes had reached the Hardy–Weinberg Equilibrium (HWE) (Table 2). G/A polymorphism was found in 77 (48%) of the study population, with 31 (40%) having hypothyroidism (Table 3). The distribution of the A/A genotype (in the cases and control groups, respectively) was 33% and 66% in the dominant model, compared to the reference genotype X*/G; which was 43% and 56% (OR = 1.53, CI = 0.54–4.26, P = 0.42). In the recessive model, the A/X* genotype was distributed at 25% and 75% compared to the reference genotype G/Gat 48% and 51% (OR = 2.8, CI = 1.04–7.60, P = 0.04) (Table 3).

Table 1 Baseline Characteristics of the Control and Hypothyroidism Subjects

Variables		Control (91)	Hypothyroidism (67)	P-value
Gender	Male	25 (100%)	0 (0.0%)	0.000*
	Female	26 (28.0%)	67 (72.0%)	
Age		27.82 ± 5.759	36.93 ± 14.656	0.000*
Weight		71.62 ± 13.481	70.59 ± 25.765	0.747
Height(cm)		1.6805 ± 0.06636	1.3616 ± 0.50993	0.000*
BMI		25.3111 ± 4.28554	28.1379 ± 15.67355	0.103

Notes: Data are mean ± SD or percentages. *Highly Significant.

Table 2 The Expected Frequencies of UCP2-868 G/A Consistency with Hardy–Weinberg Equilibrium (HWE)

Genotype	Observed	Expected	χ^2 (P-value)
A/A	21	22	0.2316 (0.8907)
G/A	77	74	
G/G	60	62	

Notes: χ^2–test with 2 degrees of freedom (If P<0.05 – not consistent with HWE).

Table 3 Genotype and Allele Distribution of the UCP2-868 G/A Polymorphism Among Hypothyroid Patients and Healthy Control Subjects

	Total n(%)	Control n(%)	Hypothyroidism n(%)	Odds Ratio (95% CI), P
Codominant Modal				
A/A	21(13.3%)	14(15.4%)	7(10.4%)	1.871(0.662–5.288), 0.237
G/A	77(48.7%)	46(50.5%)	31(46.3%)	1.388(0.703–2.743),0.345
G/G	60(38.0%)	31(34.1%)	29(43.3%)	1
Total	158(100.0%)	91(100.0%)	67(100.0%)	
Dominant Modal				
A/A	21(23.9%)	14(26.9%)	7(19.4%)	1.53(0.546–4.267)0.420
*X/G	67(76.1%)	38(73.1%)	29(80.6%)	1
Total	88(100.0%)	52(100.0%)	36(100.0%)	
Recessive Modal				
A/X*	28(31.8%)	21(40.4%)	7(19.4%)	2.806(1.039–7.583)0.042
G/G	60(68.2%)	31(59.6%)	29(80.6%)	1
Total	88(100.0%)	52(100.0%)	36(100.0%)	
Alleles				
A	98(41.7%)	60(43.8%)	38(38.8%)	1.23(0.725–2.087)0.442
G	137(58.3%)	77(56.2%)	60(61.2%)	1
Total	235(100.0%)	137(100.0%)	98(100.0%)	

Note: *Where X can be A or G.
Abbreviations: CI, confidence interval; P, P-value.

As a result, the logistic regression analysis was statistically significant only in the recessive model only; A/X* were 3 times more likely than G/G to have hypothyroidism.

Discussion

AHT is one of the most common abnormalities affecting endocrine glands worldwide, this study found a link between AHT and UCP2–866 G/A in a Saudi population from the Jazan region, where A/X* were more likely to have hypothyroidism than G/G, indicating that the –866 G allele of UCP2 reduced AHT incidence in the study population.

In general, the gender variation in the disease is significant, with females having up to 10 times the prevalence, and the prevalence increases in women as they age.[15] In addition to other factors, genetic factors contribute to the development of ATH; however, the exact mechanism that contributes to increased susceptibility of this disease remains unknown.[16,17] Because UCP2 plays an important role in maintaining cellular health, abnormalities in their function have been linked to a variety of diseases including type 2 diabetes, heart diseases, renal vascular damage, and inflammatory response.[18–22]

The data presented here show that AHT is significantly affected by gender, which is due to an extremely low number of male patients in the AHT group, and this is consistent with a previous study in the same area.[23]

Since several factors have been linked with AHT, such as gender, this is the first report to our knowledge that reports a link between UCP2–866 G/A polymorphism and AHT. A link between AHT and UCP2 mitochondrial protein has not yet been established. However, several studies have suggested that this protein may play a role in the regulation of human immune function. Previous studies on polycystic ovary syndrome (PCOS) in Saudi females coincide with our findings, where there is a significant association between the presence of the A allele and PCOS in Saudi females.[24] Several studies, however, show that the–866A allele was associated with a lower risk of diseases such as coronary artery disease,[25] chronic inflammatory diseases such as rheumatoid arthritis and systemic lupus erythematosus,[13] and type 2 diabetes.[26]

Although this study has some limitations, such as the sample size for this analysis being insufficient to draw firm conclusions about the relationship between AHT and UCP2–866 G/A, it does provide insight into the possible role of the UCP2 in disease progression, which can be validated by conducting additional studies with a larger sample size and different ethnic groups.

In conclusion, this work highlights the relation between the AHT and UCP2–866 G/A using PCR (polymerase chain reaction). The study discovered a significant link between this polymorphism and AHT, with the carrier of the –866 A allele UCP2 being 3 times more likely to have hypothyroidism than the wild-type.

Disclosure

The authors report no conflicts of interest in this work.

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