BENHA MEDICAL JOURNAL bmj
Print ISSN 1110-208X.
Online ISSN 2357-0016

Original article

Uncoupling Protein 2 and Dynamin-Related Protein1 mRNA Gene Expression as Possible Key Markers of Activity and Severity of Vitiligo

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Received: 29 February 2024

Accepted: 5 July 2024

Abstract:

Background: Vitiligo is a depigmenting skin condition due to the attenuation of melanin in the affected skin area due to specific melanocyte loss. The pathophysiology of vitiligo has been linked to both mitochondrial malfunction and oxidative stress. Dynamin-related protein 1 (DRP1) is a regulator of mitochondrial fission. Uncoupling Protein 2 (UCP2) is an essential mitochondrial protein that regulates cellular energy metabolism. **Objectives:** This study aimed to assess the gene expression levels of UCP2 and DRP1 in vitiligo patients and correlate those levels with the activity and severity of the disease. Methods: This case-control study included 40 vitiligo patients and 40 age and sex matched healthy controls. Gene expression levels of UCP2 and Drp1 were measured using an enzyme-linked immunosorbent assay. **Results:** The vitiligo group had significantly higher serum UCP2 and Drp1 gene expression levels ($p<0.001$ for each) When compared to the control group. UCP2 gene expression showed a significant negative correlation with VIDA score ($p<0.001$). Drp1 gene expression showed significant negative correlations with age and duration of vitiligo ($p=0.002$, 0.001 respectively) and a positive correlation with VIDA score ($p<0.001$). **Conclusions:** The findings suggest the potential role of dysregulation of Drp1 and UCP2 gene expression in vitiligo. For patients with vitiligo, both markers showed prognostic and diagnostic values.

Keywords: Uncoupling Protein 2; Dynamin-related protein 1; Vitiligo.

Introduction

Vitiligo is the most frequent depigmenting condition of the skin clinically characterized by white patches arising from the selective death of melanocytes. It affects 0.1%-2% of the global population [1]. Vitiligo is a complicated disorder accompanied by genetic and environmental factors together with metabolic, oxidative stress and cell detachment changes. Of note, the convergence theory, that combines immunological and biochemical factors in genetically predisposed cases, has been proposed as a unifying approach to understanding the pathophysiology of vitiligo because neither individual mechanism can adequately account for all aspects of this complex disorder [2].

Numerous studies have highlighted the importance of certain genes and pathways in disease susceptibility and pathogenesis in the development of vitiligo, which has long been linked to genetic factors [3]. The discovery of valid genetic markers for vitiligo might facilitate early diagnosis, risk assessment, and the development of focused treatment strategies. There is an increasing interest in studying the mRNA expression levels of certain genes as possible genetic markers for vitiligo [4,5].

UCP2 isan essential mitochondrial protein that regulates cellular energy metabolism. It participates in the decoupling of oxidative phosphorylation, which might enhance the formation of reactive oxygen species (ROS) [6,8]. Mitochondria constantly undergo fission and fusion, adjusted by a group of major GTPases called DRPs. Mitochondrial fission mediated by DRP1 is essential for cellular functions including apoptosis, mitophagy, and energy consumption [9,10]. Recent data shows the gene expressions of such mitochondrial regulatory proteins may be accompanied by autoimmune disorders which include vitiligo [8].

In this study, we investigate the mRNA expression levels of UCP2 and DRP1 as potential genetic markers for vitiligo.

Subjects and Methods:

Study population

This case-control study included 40 vitiligo patients and 40 healthy controls of the same age and sex. The patients were selected from the outpatient clinic of the Department of Dermatology and Venereology at Benha University Hospital in Egypt in the period between June 2022 to November 2022. Patients of varied sexes and ages with variable degrees of vitiligo severity were included in the research.

Patients were diagnosed clinically and underwent Wood's lamp examination for detection of the clinical type of vitiligo. Patients below 16 years old, patients more than 50 years old, patients with inflammatory or other autoimmune skin diseases, patients who were taking any systemic medications or receiving phototherapy that might affect vitiligo, pregnant women, and lactating mothers were also excluded from the study.

A full history was taken from all patients with vitiligo Tayeb and Picardo [11]. The disease severity was evaluated and classified using the Vitiligo Area Severity Index score, which is equal to all body sites (hand units) × residual depigmentation [12].

The disease activity was assessed using the Vitiligo Disease Activity Score. The grading was as follows: +4, activity of 6 weeks or less length; +3, activity of six weeks to 3 months; +2, activity of 3-6 months; +1, activity of 6-12 months; zero, stable for one year or more; and -1, stable with spontaneous regimentation for at least one year [13].

Laboratory investigations:

Blood samples were collected from all subjects. A volume of 5 ml of venous blood was obtained in sterile vacuum blood-collecting tubes. The serum was separated by centrifugation, and the samples were stored at <-20°C. The serum levels of UCP2 and DRP1 were measured using ELISA kits that employed a double-

antibody sandwich technique to detect the respective proteins in the serum samples specific to each protein. The ELISA kits used were the Human UCP2 ELISA Kit; Evelop company Catalog No: DL-UCP2-Hu. Detection Range: 0.156-10ng/mL. The minimum detectable level of UCP2 is less than 0.051ng/mL and Human Dynamin 1 ELISA kit; Evelop company Catalog No: DL-DNM1-Hu. Detection Range: 0.312-20ng/ml. The minimum detectable level of DNM1 is less than 0.112ng/ml.

Ethical considerations:

The study's protocol was approved by the Ethical Scientific Committee of Benha University {M.S 18.6.2022}. Before enrollment in the study, the patients were fully explained to all study procedures and were asked for their informed consent.

Statistical analysis:

SPSS V. 25.0 was utilized for the statistical analysis. Using the Student T-Test, the statistical significance of the variation in the parametric variable between the two study groups was determined. A correlation analysis was used to assess the degree of relationship between two quantitative variables. A helpful tool for assessing the sensitivity, specificity, PPV, and NPV of quantitative measurements that divide instances into two categories is the ROC Curve. Generalized linear models were utilized in both logistic and linear regression analysis to predict risk variables. A P-value that is equal to or less than 0.05 was significant in statistical terms.

Results

The mean age of the studied vitiligo patients was 28.9 years ± SD 10.53 and regarding sex 77.5% were males and 22.5% were females. The control group was 40 of matched age and sex ($p \geq 0.05$). The study showed 32.5% of patients with vitiligo had a family history of vitiligo. The course of vitiligo was of progressive course in 25 (62.5%) patients while 9 (22.5%) patients were complaining of a stationary course, and (15%) were regressive (table 1).

The study showed the vitiligo group had a statistically significant difference in serum UCP2 and Drp1 gene expression levels ($p<0.001$ for each) When compared to the control group ($p<0.001$) for each. The ROC revealed that UCP2 gene expression showed a high accuracy AUC (0.978) as a diagnostic ability for vitiligo. Sensitivity was 97.5%, specificity was 82.5%, PPV was 84.78%, NPV was 97.06%, and accuracy was 90%. In addition, Drp1 gene expression showed a high accuracy AUC (0.986) as a diagnostic ability for vitiligo. Sensitivity was 100%, specificity was 85%, PPV was 86.96%, NPV was 100%, and accuracy was 92.5% (table 2 and Fig 1).

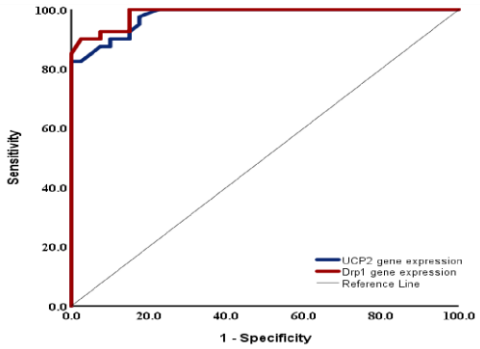

Figure 1. ROC of UCP2 and Drp1 for discrimination between vitiligo patients and control group.

Table 1. Clinical data of vitiligo among vitiligo patients.

	Vitiligo n = 40 patients	
	n	%
Family history		
Negative	27	67.5
Positive	13	32.5
Course of disease		
Stationary	9	22.5
Progressive	25	62.5
Regressive	6	15.0
Activity of disease		
Stable	15	37.5
Active	25	62.5
Clinical type		
Vulgaris	19	47.5
Focal	10	25.0
Acral	6	15.0
Acrofacial	5	12.5
VIDA Score		
Mean ± SE.	2.30 ± 0.29	
Median (Range)	3.0 (-1.0 – 4.0)	
VASI Score		
Mean ± SE.	2.93 ± 0.41	
Median (Range)	2.0 (0.50 – 12.0)	

VASI, vitiligo area severity index; VES, vitiligo extent score; VIDA, vitiligo disease activity score.
Numerical parameters were compared using the Mann-Whitney test. To compare numerical data, the t-test was used, which expresses the mean and standard deviation; for categorical data, the chi square test was used, which expresses the number and percentage.

Table 2. Validity of UCP2 and Drp1 for discrimination between vitiligo patients and control group**.**

AUC	95% CI	p	Cut off	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
UCP2 gene expression								
0.978	0.954 – 1.000	<0.001*	<0.915	97.5	82.5	84.78	97.06	90.0
Drp1 gene expression								
0.986	0.969 – 1.000	<0.001*	>1.625	100.0	85.0	86.96	100.0	92.5

CI, confidence interval; *: Significant ≤0.05

The present study showed a significant negative correlation between UCP2 and Drp1 gene expression (Fig 2).

The study showed a significant association between UCP2 gene expression and the course of disease ($p<0.05$). The UCP2 in stationary and regressive was statistically significantly higher than progressive. Regarding the activity of the disease, the result showed the UCP2 in unstable disease was statistically significantly lower than that in stable cases ($p<0.05$). UCP2 gene expression serum level showed a significant negative correlation with VIDA score ($p<0.001$) and a significant positive correlation with age and duration of disease. While no significant correlation was found regarding VASI score (table 3).

Drp1 gene expression showed significant negative correlations with age and duration of vitiligo ($p=0.002$, 0.001 respectively) and a positive correlation with VIDA score ($p<0.001$). The median Drp1 gene expression level in progressive cases was

higher than that in stationary or regressive cases. The results showed a statistically significant association between Drp1 and activity of the disease, the median Drp1 in unstable disease was higher than that in stable ($p<0.05$). No significant correlation was found with VASI score (table 4).

Table 3. Correlation between UCP2 gene expression and different parameters among vitiligo patients.

	UCP2 gene expression	
	r	p
Age (years)	0.464	0.003*
Duration of disease (months)	0.508	0.001*
VIDA Score	-0.957	<0.001*
VASI Score	-0.238	0.140

r, Spearman correlation coefficient; *: Significant ≤0.05

Table 4. Correlation between DRP1 gene expression and different parameters among vitiligo patients

	DRP1 gene expression	
	r	p
Age (years)	-0.477	0.002*
Duration of disease (months)	-0.503	0.001*
VIDA Score	0.960	<0.001*
VASI Score	0.251	0.118

r, Spearman correlation coefficient; *: Significant ≤0.05

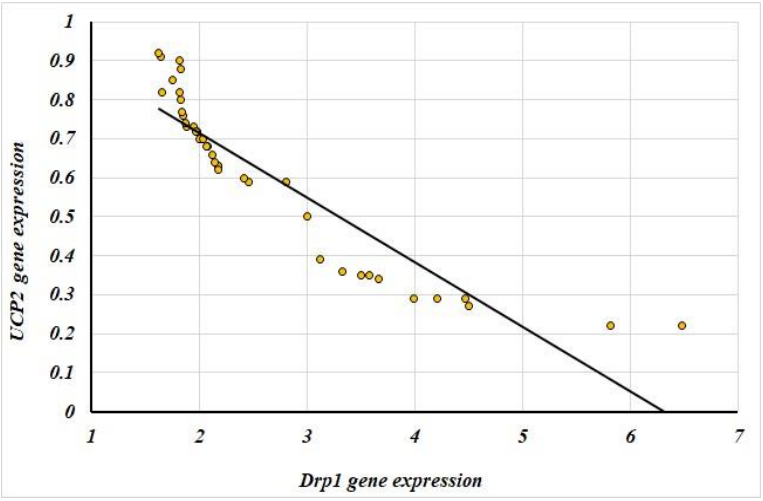

Figure 2. Correlation between UCP2 and Drp1 gene expression among vitiligo patients.

Discussion

Vitiligo is now well recognized as an autoimmune condition related to several causes such as metabolism, oxidative stress, cellular detachment, genetic predisposition, and environmental influences. The psychological impact of vitiligo can significantly affect a person's daily life [7, 14].

As a member of the uncoupling protein family, UCP2 is an inner mitochondrial membrane protein that is crucial in reducing the potential of the mitochondrial membrane and releasing metabolic energy while preventing the buildup of oxidative stress [15, 16].

DRPs, A set of big GTPases (Drps) drive the ongoing fission and fusion processes of mitochondria. Drp1 is the principal mediator of mitochondrial fission; it is located in the cytoplasm and is drawn to specific sites on the mitochondrial surface

for division [17,18].

Also, some researchers [19] stated that Mitochondria control energy metabolism and cellular activity. Which is adjusted by a group of major GTPases called Drps. The main regulator of mitochondrial fission is Drp1, which has essential role in the context of inflammatory skin disorders [20].

Considering that autoimmune diseases are strongly related to mitochondrial regulatory proteins. The expression of these proteins, such as UCP2 and Drp1, may be related to autoimmune illnesses such as vitiligo [21]. Therefore, this case-control study aimed to assess the levels of UCP2 and Drp1 gene expression in the serum of forty vitiligo patients selected from the Dermatology and Venereology Department at Benha University Hospitals. The research also included 40 age and sex-matched healthy controls. The aim was to detect changes in UCP2 and DRP1 gene expression levels in the plasma of vitiligo patients and to detect the relation between their levels and vitiligo disease severity and activity.

In the current study, Vitiligo patients had substantially lower gene expression levels of UCP2 compared to healthy controls ($p<0.001$). UCP2, an inner mitochondrial membrane protein, is critical for maintaining mitochondrial membrane potential and minimizing oxidative stress accumulation. Therefore, decreased UCP2 expression may contribute to increased oxidative stress inside the melanocytes, resulting in their depletion and eventual depigmentation in vitiligo [8, 22]. The significant difference of UCP2 and Drp1 gene expression levels in vitiligo patients when compared to control subjects, together with UCP2 gene expression sensitivity was 97.5%, specificity was 82.5% at a cutoff value < 0. 915 and accuracy was 90% suggesting its diagnostic ability for vitiligo. In addition, Drp1 gene expression showed a high accuracy (92.5%) as a diagnostic ability for vitiligo at best cut-off value >1.625, sensitivity was 100%, and specificity was 85%. In addition, the elevation of Drp1 gene expression in vitiligo patients suggests an accelerated mitochondrial fission process [23,24].

This study's correlation analysisrevealed a strong relationship between the levels of gene expression for UCP2 and Drp1 and the degree and activity of vitiligo. UCP2 gene expression had a significant negative correlation with VIDA score and a significant positive correlation with age and duration of disease suggesting a relationship between mitochondrial malfunction and the development of vitiligo. While Drp1 gene expression showed significant negative correlations with age and duration of vitiligo and a positive correlation with VIDA score suggesting that enhanced mitochondrial fission may be linked to the active stages of vitiligo.

Conclusion

In conclusion, this case-control study provides evidence of altered gene expression levels of UCP2 and Drp1 in vitiligo patients. The downregulation of UCP2 and upregulation of Drp1 suggest mitochondrial dysfunction and dysregulated mitochondrial dynamics in vitiligo pathogenesis markers for vitiligo. Both markers had diagnostic and prognostic values for vitiligo patients. Further research in this area with a larger sample size could contribute to the development of targeted therapeutic approaches and personalized medicine for vitiligo patients.

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To cite this article: Maha M. Osman, Amany I. Mustafa, Karim R. Karim, Fatma M. Elesawy. Uncoupling Protein 2 and Dynamin-Related Protein1 mRNA Gene Expression as Possible Key Markers of Activity and Severity of Vitiligo. BMFJ 2024;41(8):463-469.