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Evaluation of mRNA expression level of the ATP synthase membrane subunit c locus 1 (*ATP5G1*) gene in patients with schizophrenia

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ABSTRACT

Background: Schizophrenia is a serious, complex mental disorder. The impairment of oxidative phosphorylation has a detrimental consequence on CNS function. Different ATP synthase subunits have been involved in the pathological process of various neurodegenerative disorders. Our goal was to evaluate the mRNA expression level of the ATP synthase membrane subunit c locus 1 (*ATP5G1,* also named *ATP5MC1*) gene in patients with schizophrenia.

Methods: Determination of the expression levels of *ATP5G1* in plasma and peripheral blood mononuclear cells (PBMCs) were performed by real-time PCR in 90 controls and 90 patients with schizophrenia.

Results: Patients had significantly decreased *ATP5G1* mRNA expression levels in both plasma and PBMCs compared to controls. The receiver operating characteristic curve was applied to detect a cut-off value of *ATP5G1* expression in plasma and PBMCs. The *ATP5G1* relative expression in PBMCs had better performance with a cut-off value $\leq$ 21 (AUC = 0.892, P < 0.001), sensitivity of 94.44%, and specificity of 72.22% in discriminating between schizophrenic patients. *ATP5G1* expression in PBMCs was an independent predictor in schizophrenia.

Conclusion: This study revealed a down-regulation of *ATP5G1* expression in schizophrenia, precisely expression in PBMCs. That might give insight into the role of *ATP5G1* gene in the pathogenesis of schizophrenia.

1. Introduction

Schizophrenia is a serious complex mental disorder with a prevalence rate around 1% of the population globally. Schizophrenia has three groups of manifestations divided into negative, positive, and cognitive symptoms [1]. Despite positive symptoms like delusions and hallucinations are the main characterizations in schizophrenia, negative symptoms like social isolation and apathy are considered major contributing factors to the patient's performance and quality of life [2]. Various theories have been proposed for the development and progression of schizophrenia, including an interaction between genetic risk factors and different environmental components [3].

Adenosine triphosphate (ATP) availability is a prime requirement for central nervous system (CNS) function as brain activity relies significantly on ATP feed [4]. In patients with schizophrenia, several imaging workups exposed distorted metabolism, as stated by alteration in glucose and ATP in various brain areas [5]. Additionally, the severity of negative symptoms and psychological manifestations are related to ATP levels [4]. ATP is basically produced in mitochondria by oxidative phosphorylation, and it generates approximately ninety percentage of ATP required for neurological functions and cellular signaling in the brain. Therefore, the impairment of oxidative phosphorylation has a detrimental consequence on CNS function and neurological performance [6]. Moreover, mitochondria and oxidative phosphorylation are involved in several cellular pathways such as inflammation, oxidative stress, and cellular apoptosis [7].

The oxidative phosphorylation or the electron transport chain contains five protein complexes within the inner mitochondrial membrane [8]. The ATP synthase, or F1/Fo, is the fifth constituent of this chain. It entails two subunits, the membrane-traversing subunit (Fo) and (F1)

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subunit within the mitochondrial matrix [9]. The Fo subunit involves nine polypeptides (a, b, c, d, e, f, g, F6 and 8), whereas the F1 complex contains five components (α, β, γ, δ and ε) [10]. Fo complex's main three components (a, b, and c) are diversely coded. The (a) polypeptide is coded via mitochondrial DNA, while b and c are coded via nuclear DNA. Three distinctive genes encode the c subunit, ATP synthase membrane subunit c locus 1, 2, and 3 *(ATP5G1* [also named *ATP5MC1], ATP5G2*, and *ATP5G3*, respectively) [11].

Different ATP synthase subunits have been involved in the pathological process of various neurodegenerative and developmental disorders [12]. Also, irregularity in oxidative phosphorylation was related to the progression of depressive manifestation [13]. The *ATP5G1* gene had not been thoroughly investigated in schizophrenia. Therefore, in this analysis, we aimed to evaluate the mRNA expression level of the *ATP5G1(*or *ATP5MC1)* gene in patients with schizophrenia and its potential value in diseases progression.

2. Materials and methods

This research was carried out at Medical Biochemistry and Molecular Biology department with the aid of Neuropsychiatry and Medical Physiology departments, faculty of Medicine, Menoufia University. This analysis enrolled 180 subjects: 90 patients with schizophrenia (newly diagnosed and before starting therapy) and 90 age and gender-matched healthy controls.

Diagnosis of schizophrenia was dependent upon the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition, (DSM-5), which states that patients must have experienced at least 2 of these mentioned symptoms: (Delusions, Hallucinations, Disorganized speech, Disorganized or catatonic behavior, and negative symptoms) for at least six months to be considered as schizophrenic. During this period, the patient must experience active symptoms at least for one month with social or occupational deterioration problems lasting for a significant amount of time [14]. Schizoaffective disorder, depressive and bipolar disorder with psychotic features and substance addictions have been ruled out Positive And Negative Syndrome Scale (PANSS) is a psychological scale, that had been determined to measure schizophrenic patients' symptoms [15]. Regarding our patients, 88 out of 90 schizophrenic subjects had positive symptoms in the form of: 37 suffered from delusions, hallucinatory behaviors, suspiciousness and hostility, 31 had delusions, hallucinatory behaviors, suspiciousness and conceptual disorganization and the rest 20 patients complained from delusions, suspiciousness and grandiose. 57 out of 90 patients had negative symptoms. 27 of them had passivity, apathetic and social withdrawal, 16 had poor rapport, blunted affect and difficult abstract thinking and the last 14 cases suffered from emotional withdrawal, poor rapport and stereotype thinking.

The study followed the Helsinki Declaration directions. All patients and controls provided informed consent. The analysis protocol was accepted from the Ethics Committee of the Faculty of Medicine, Menoufia University.

For all participants, we performed a complete history taking and a full clinical examination. Laboratory investigations included measurement of lipid profile [total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-c), and estimation of low-density lipoprotein cholesterol (LDL-c)] and determination of *ATP5G1* mRNA expression was performed by real-time q PCR technique.

2.1. Samples collection

Ten ml of blood (venous) were withdrawn from each sharer and used as follows:

- 3 ml were hold in plain tube, stood at room temperature for 30 min to clot and then centrifuged for 10 min at 4000 r.p.m. The obtained serum was stored at −80 °C.
- 7 ml were divided into 2 EDTA containing tubes. One tube was for isolation of peripheral blood mononuclear cells by Histopaque −1077, Sigma-Aldrich, UK According to the manufacturer's directions. The PBMCs layer isolation was done within 8 h of the blood collection. The other EDTA tube was used for plasma separation. The separated plasma and PBMCs layers were stored at −80 °C till RNA extraction to determine *ATP5G1* gene expression by RT-qPCR.

2.2. Assay methods

Serum TC, TG and HDL-c were assayed by the enzymatic colorimetric test, using Bio Merieux kit, France. Serum LDL-c was calculated by friedewald equation as LDL-c = (TC) – [(HDL-c + (TG/5))] in mg/dl [16].

2.3. Estimation of gene expression

RNA was isolated from both PBMCs and plasma of each subject using QIAamp RNA Blood Mini Kit (Qiagen, USA, 2013). The quality and quantity of extracted RNA was confirmed using nanodrop spectrophotometer (NanodropTechnologies). Complementary DNA was synthesized using QuantiTect Reverse Transcription Kit (Qiagen, Applied Biosystems, USA, 2012). Then real-time PCR was performed using Quanti Tect SYBR Green PCR Kit with ready-made Quanti Tect Primer Assay, Qiagen. The used primers' accuracy was confirmed utilizing the Primer BLAST. Each Primer (25 nmole) was dissolved in 250 μl RNase-free water to obtain solution of 100 μmol/L as a final concentration. The following primers were used for measurement of *ATP5G1* mRNA levels in both plasma and PBMCs: forward and reverse primers of human *ATP5G1*, 5′- GGGTAGTAGGAGTGCAGACTGA -3′and 5′- GGGCTATTCAAGAAGGAGGCA -3′, respectively. Concerning *β-actin* the forward and reverse primers were 5′-TCCCTGGAGAAGAGCTACGA-3′and 5′-TGAAGGTAGTTTCGTGGATGC-3′, respectively. The PCR reaction was performed in a net volume of 20 μL, containing 10 μL SYBR Green 2x QuantiTect PCR Master Mix, 3 μL of cDNA, 1 μL for each forward and reverse primers and 5 μl of RNase-free water. The mixture was incubated for 3 min at 94 °C then followed by 60 cycles; denaturation for 30 s at 94 °C, annealing for 40 s at 55 °C, and extension for 31 s at 72 °C. Data analysis was done in Applied Biosystems 7500 software version 2.0.1. The mRNA level of housekeeping gene *β-actin* was used to normalize *ATP5G1* mRNA. The relative quantification (RQ) of gene expression was done using the comparative ΔΔCt method.

2.4. Statistical analysis

The IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp) was used in data analysis. We utilized the Kolmogorov- Smirnov test to validate the normality of distribution of variables. The categorical variables were evaluated in our groups by the Chi-square test. Mann Whitney test was used to compare between two groups for not normally distributed quantitative variables. Spearman coefficient was used to correlate between two distributed abnormally quantitative variables. Receiver operating characteristic curve (ROC) was generated by plotting sensitivity (TP) on Y axis versus 1-specificity (FP) on X axis at different cut off values. The area under the ROC curve denotes the diagnostic performance of the test. Area more than 50% gives acceptable performance and area about 100% is the best performance for the test. The ROC curve in this analysis determines the performance of the relative quantification (RQ) of *ATP5G1* mRNA to discriminate between patients and controls. Logistic regression was used to identify the independent factors for Schizophrenia. The significance of the extracted results was judged at the 5% level.

3. Results

This analysis involved 90 schizophrenic patients (38 males and 52

females) with ages ranging from 18 to 58 and 90 healthy subjects as controls (50 males and 40 females) with ranged ages from 25 to 68. The age and sex did not differ significantly between the patients and controls. The clinical characteristics of our patients revealed that the majority of the patients (97.8%) reported positive symptoms More than half (63.3%) of the patients showed negative symptoms, while only 31 (34.4%) suffered from cognitive symptoms. Evaluating the investigated laboratory parameters revealed a significant elevation in LDL-c and decreased HDL-c concentrations in patients compared to controls. Serum concentrations of TC and TG were higher in patients than controls, but variation was insignificant. Assessment of the relative *ATP5G1* expression showed that the patients had significantly decreased *ATP5G1* mRNA levels in both plasma and PBMCs compared to the control group (Table 1).

As to determine the performance of the relative quantification (RQ) of *ATP5G1* mRNA to discriminate between patients and controls, the ROC curve was used to assess the performance of *ATP5G1* expression. The *ATP5G1* relative expression in PBMCs had the best achievement with a cut-off value ≤ 21 (AUC = 0.892, $P < 0.001$), the sensitivity of 94.44%, and specificity of 72.22%. However, the *ATP5G1* expression level in plasma reported only a sensitivity of 72.22% and specificity of 66.67% (AUC = 0.764, cut-off value ≤ 2.9, $P < 0.001$) (Table 2; Fig. 1).

Table 1
Comparison between the patients and controls according to different parameters.

	Patients (n = 90)	Controls (n = 90)	Test of Sig.	P
Age (years)				
Mean ± SD.	34.7 ± 9.3	36.1 ± 12.5	U=	0.683
Median (Min. – Max.)	33 (18–58)	32 (25–68)	3907.500	
Sex				
Male	38 (42.2%)	50 (55.6%)	χ^2=	0.074
Female	52 (57.8%)	40 (44.4%)	3.202	
Positive symptoms	88 (97.8%)	0 (0%)	χ^2 = 172.17*	<0.001*
Negative symptoms	57 (63.3%)	0 (0%)	χ^2 = 83.415*	<0.001*
Cognitive symptoms	31 (34.4%)	0 (0%)	χ^2 = 37.450*	<0.001*
HDL-c				
Mean ± SD.	45.4 ± 7.9	60.2 ± 8.5	U=	<0.001*
Median (Min. – Max).	43.7 (32.2–69.1)	62.6 (42.3–73.9)	905.0*	
LDL-c				
Mean ± SD.	147.2 ± 54	101.3 ± 17.2	U=	<0.001*
Median (Min. – Max.)	178.5 (70–226.3)	106.4 (65.6–133.9)	2631.0*	
TC				
Mean ± SD.	217.5 ± 66.6	180.7 ± 13.1	U=	0.122
Median (Min. – Max.)	262.5 (137.3–298.9)	181 (154.9–198.4)	3510.0	
TG				
Mean ± SD.	129.2 ± 69.8	100.8 ± 24.6	U=	0.070
Median (Min. – Max.)	168.2 (37.5–225.2)	93.3 (62.5–151.9)	3417.0	
Suicide				
No	48 (53.3%)	90 (100%)	χ^2=	<0.001*
Yes	42 (46.7%)	0 (0%)	54.783*	
***ATP5G1* expression level In Plasma**				
Mean ± SD.	2.37 ± 0.76	3.34 ± 1.15	U=	<0.001*
Median (Min. – Max.)	2.50 (0.65–3.79)	3.11 (0.75–5.88)	1913.5*	
In PBMCs				
Mean ± SD.	9.41 ± 6.19	26.16 ± 11.26	U=	<0.001*
Median (Min. – Max.)	8.80 (0.70–24.99)	25.34 (0.93–49.72)	872.50*	

χ^2: Chi square U: Mann Whitney.
p: p value for comparing between the two studied groups.
*: Statistically significant at $p \leq 0.05$.

Regarding schizophrenic patients and to assess the association of our gene expression with disease indicators of laboratory findings and clinical manifestations, Table 3 specifies significant positive correlations in the relative expression of *ATP5G1* in both plasma and PBMCs with serum HDL-c levels ($r = 0.262$, $P = 0.012$ and $r = 0.359$, $P = 0.001$ respectively). The LDL-c levels negatively correlated with both *ATP5G1* expression in plasma ($r = -0.209$, $P = 0.048$) and expression in PBMCs ($r = -0.280$, $P = 0.008$). Additionally, as expected, plasma *ATG5G1* expression level was positively correlated with their expression level in PBMCs ($r = 0.840$, $P < 0.001$). No significant correlations between gene expression and age, TC or TG in our patients were detected.

Reduced *ATP5G1* expression levels in both plasma and PBMCs were significantly more prevalent in patients with negative ($P < 0.005$ and $P < 0.001$ respectively) or suicidal ($P = 0.039$ and $P = 0.023$ respectively) symptoms. Additionally, the *ATP5G1* expression level in PBMCs was markedly down-regulated ($P < 0.005$) in patients with cognitive symptoms than those without such manifestations, which may indicates a possible effect on disease progression. There were no significant relations between *ATP5G1* expression level and sex or the presence of positive symptoms (Table 4).

Moreover, to confirm the impact of gene expression on schizophrenia and discard the possibility that the observed reduced levels of expression are due to depression, we compared the *ATP5G1* expression level in the patients without negative or suicidal symptoms to controls and revealed significantly decreased expression in these patients in both plasma (U = 456.0, $P = 0.045$) and PMNCs (U = 286.50, $P < 0.001$) (Fig. 2).

As presented in Table 5, the multivariate logistic regression analysis shown that *ATP5G1* relative expression in PBMCs ($P < 0.001$, OR 0.800, 95% 0.727–0.880) and HDL-c ($P = 0.001$, OR 0.810, 95% 0.719–0.912) were independent risk factors for schizophrenia.

4. Discussion

Schizophrenia is a serious, markedly diverse psychiatric disorder with various manifestations. Although the expanded incidence rate and incapacitating consequences of schizophrenia, little is available regarding its pathogenesis [17]. This disorder has a heterogonous genetic nature, and gene expression evaluation may expose the association between altered expressions and the pathogenesis of schizophrenia [18]. Moreover, gene expression is supposed to be the chief way genotype principally affects the disease phenotype [17]. Various reports have revealed that expression aberration in PBMCs might resemble the molecular modifications in the brain [19]. Likewise, the brain and PBMCs may share a similar mRNA expression profile [20]. Previous investigations have found that altered gene expressions in PBMCs specimens may be implicated in the pathogenesis of schizophrenia [17]. Additionally, circulating cell-free nucleic acids obtain a pronounced value as a potential biomarker for different diseases [21]. Mitochondrial dysfunction and subsequent altered cellular bioenergetics are reasonable hypotheses for clarifying the pathogenesis of schizophrenia [22].

In the current analysis, our goal was to evaluate the expression pattern and the potential value of *ATP5G1 (*or *ATP5MC1)* in both PBMCs and plasma in patients with schizophrenia. We found a down-regulated *ATP5G*1 relative expression in both PBMCs and plasma in patients with schizophrenia than controls. The expression level in PBMCs showed better performance in discrimination between patients and controls by ROC curve than their expression in plasma. Additionally, expression in PBMCs was an independent risk factor for schizophrenia.

These findings align with the results of **Zeng et al.,** study [23] which carried out on major depressive disorders. They revealed a significant drop in the *ATP5G1* mRNA level in PBMCs as compared to healthy controls. Furthermore, they reported a significant differential expression and methylation profile of *ATP5G1* in brain areas. The *ATP5G1* encodes the c unit of the Fo component of the ATP synthase in oxidative phosphorylation that catalyzes ATP production [24]. Additionally,

Table 2
Validity (AUC, sensitivity, specificity) *ATP5G1* expression level in plasma and PBMCs to discriminate patients (n = 90) from control (n = 90).

ATP5G1 expression level in	AUC	P	95% C.I	Cut-off	Sensitivity	Specificity	PPV	NPV
Plasma	0.764	<0.001*	0.694–0.834	≤2.9	72.22	66.67	68.4	70.6
PBMCs	0.892	<0.001*	0.839–0.946	≤21	94.44	72.22	77.3	92.9

AUC: Area Under a Curve p value: Probability value.
CI: Confidence Intervals.
NPV: Negative predictive value PPV: Positive predictive value.
*: Statistically significant at $p \leq 0.05$.

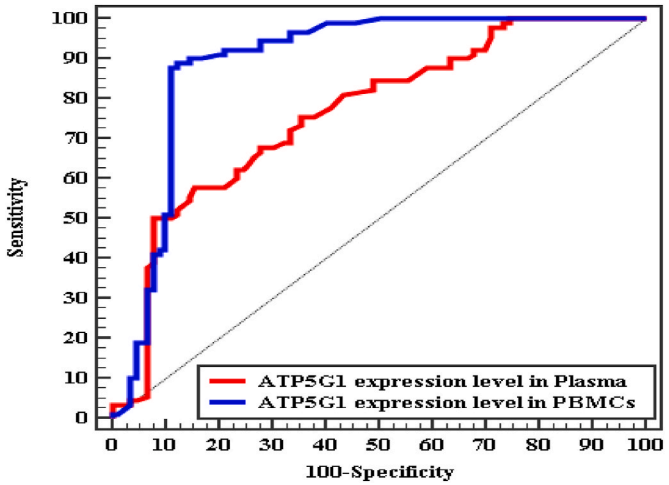

Fig. 1. ROC curve for *ATP5G1* expression level in plasma and PBMCs to discriminate patients (n = 90) from control (n = 90).

Table 3
Correlation between *ATP5G1* expression level in plasma and PBMCs and different parameters in patients with schizophrenia (n = 90).

	ATP5G1 expression level in plasma		*ATP5G1* expression level in PBMCs	
	r_s	p	r_s	p
Age (years)	0.083	0.435	0.027	0.800
HDL-c	0.262	0.012*	0.359	0.001*
LDL-c	−0.209	0.048*	−0.280	0.008*
TC	−0.089	0.403	−0.150	0.158
TG	0.043	0.688	0.004	0.968
***ATP5G1* expression level in plasma**			0.840	<0.001*

r_s: Spearman coefficient.
*: Statistically significant at $p \leq 0.05$.

Table 4
Relation between *ATP5G*1 expression level in plasma, PBMCs and different parameters in patients' group (n = 90).

	N	*ATP5G1* expression level in plasma		*ATP5G1* expression level in PBMCs	
		Median (Min. – Max.)	Mean ± SD.	Median (Min. – Max.)	Mean ± SD.
Sex					
Male	**38**	2.46 (0.70–3.79)	2.38 ± 0.78	8.89 (1.0–23.12)	9.43 ± 5.47
Female	**52**	2.50 (0.65–3.77)	2.36 ± 0.76	8.41 (0.70–24.99)	9.40 ± 6.72
U(p)		963.0 (0.838)		938.50 (0.686)	
Positive symptoms					
No	**2**	2.60 (2.32–2.87)	2.60 ± 0.39	11.05 (8.34–13.77)	11.05 ± 3.83
Yes	**88**	2.50 (0.65–3.79)	2.36 ± 0.77	8.80 (0.70–24.99)	9.38 ± 6.24
U(p)		73.0 (0.702)		69.50 (0.629)	
Negative symptoms					
No	**33**	2.80 (1.53–3.79)	2.68 ± 0.73	11.43 (2.10–24.99)	12.41 ± 6.64
Yes	**57**	2.05 (0.65–3.53)	2.19 ± 0.73	5.78 (0.70–23.09)	7.68 ± 5.23
U(p)		608.50* (0.005*)		542.0* (0.001*)	
Cognitive symptoms					
No	**59**	2.65 (0.72–3.79)	2.49 ± 0.77	9.35 (1.0–24.99)	10.61 ± 6.24
Yes	**31**	2.06 (0.65–3.21)	2.14 ± 0.71	4.93 (0.70–23.12)	7.14 ± 5.48
U (p)		697.0 (0.065)		586.50* (0.005*)	
Suicide					
No	**48**	2.71 (0.70–3.79)	2.52 ± 0.82	10.83 (1.0–24.99)	11.16 ± 7.03
Yes	**42**	2.02 (0.65–3.44)	2.19 ± 0.67	6.34 (0.70–15.01)	7.42 ± 4.34
U (p)		752.50* (0.039*)		726.0* (0.023*)	

U: Mann Whitney.
p: p value for association between ATP5G1 expression level in plasma, PBMCs and different parameters.
*: Statistically significant at $p \leq 0.05$.

Mitochondrial ATP generation was reported to be declined in both fresh and frozen olfactory neuroepithelium specimens in patients with schizophrenia compared to controls [25].

The mitochondrial electron transport chain has been recommended as a significant variable in the pathogenesis of a scope of various neuropsychiatric diseases. Also, this energetic process in schizophrenia was described in numerous brain areas and platelets [22]. Deterioration of ATP generation may be associated with the pathological process of schizophrenia, as declined bioenergetics in the brain affects cognitive functions and synaptic transmission [26].

In a previous study, **Natera-Naranjo and colleagues** [27] stated that silencing of axonal *ATP5G1* expression by small interference RNA (siRNA) caused a remarkable decline in ATP and ATP5G1 protein concentrations. Moreover, repression of *ATP5G1* mRNA increased the synthesis of reactive oxygen species (ROS). Likewise, **Vives-Bauza et al.,** reported in their study that repression or silencing of any of the three subunit c isoforms caused a deficiency in ATP production in Hela cells [28].

The mitochondria is suggested to have a significant role in producing ROS and oxidative stress in neurons [29]. Cellular dysfunction as a result of decreased ATP synthesis is correlated with the increased mitochondrial generation of ROS. Consequently, declined ATP production and increased oxidative stress are suggested as the central stimulants of distorted neurological functions [30].

The oxidative stress due to elevated ROS has been associated with schizophrenia and was related to deteriorating disease course and negative progression [31]. Additionally, amplified oxidative stress in patients with schizophrenia was related to the increased aggressiveness of the negative symptoms [32]. Additionally, the severity of negative symptoms was related with ATP levels [4]. This may explain our finding of the inverse relationship between the expression level of *ATP5G1* and the presence of negative symptoms, as supposed the lower *ATP5G1*expression will result in decreased ATP production and increased ROS.

*ATP5G1*expression was also evaluated in other disorders.

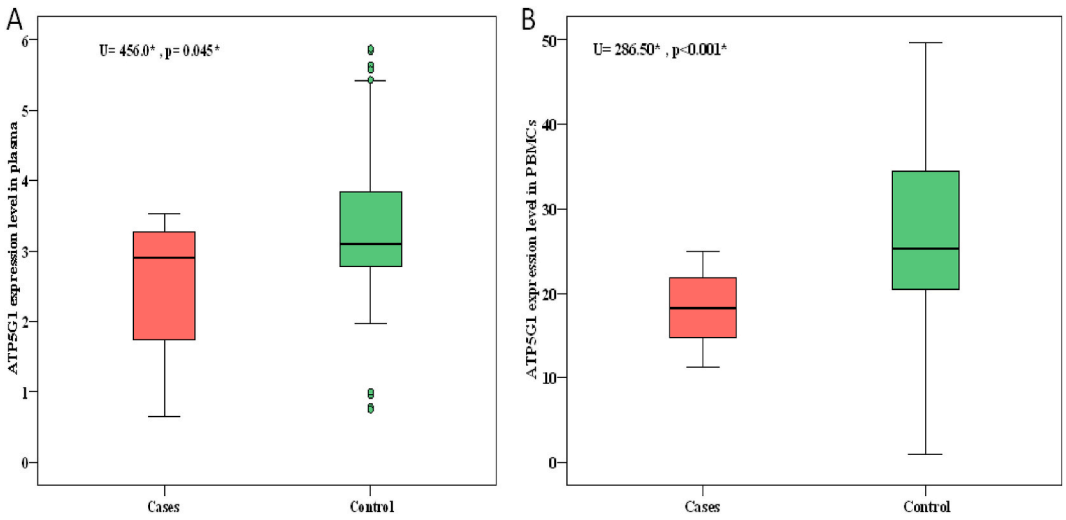

Fig. 2. A- Comparison between patients with no negative or suicide symptoms (n = 15) and controls (n = 90) regarding *ATP5G1* expression level in plasma. **B-** Comparison between patients with no negative or suicide symptoms (n = 15) and controls (n = 90) regarding *ATP5G1* expression level in PMNCs. .

Table 5
Univariate and multivariate analysis for the studied parameters affecting Schizophrenia.

	Univariate		#Multivariate	
	P	OR (95%C.I)	P	OR (95%C.I)
Sex (female)	0.074	1.711 (0.948–3.085)		
Age (years)	0.394	0.988 (0.962–1.015)		
Positive symptoms	0.995	–		
Negative symptoms	0.997	–		
Cognitive symptoms	0.998	–		
HDL-c	<0.001*	0.833 (0.793–0.874)	0.001*	0.810 (0.719–0.912)
LDL-c	<0.001*	1.028 (1.018–1.038)	0.577	0.978 (0.903–1.059)
TC	<0.001*	1.016 (1.009–1.022)	0.249	1.047 (0.968–1.133)
TG	0.001*	1.010 (1.004–1.016)	0.516	0.992 (0.966–1.017)
Suicide	0.997	–		
***ATP5G1* expression level In plasma**	<0.001*	0.318 (0.207–0.490)	0.158	1.894 (0.781–4.595)
In PBMCs	<0.001*	0.823 (0.780–0.868)	<0.001*	0.800 (0.727–0.880)

OR: Odd's ratio.
C.I: Confidence interval LL: Lower limit UL: Upper Limit.
#: All variables with $p < 0.05$ was included in the multivariate.
*: Statistically significant at $p \leq 0.05$.

Bruggemann and colleagues [33] reported down-regulation of *ATP5G1* expression in clear cell renal cell carcinoma tissues compared to normal renal tissues. Moreover, ATP5G1 protein down-regulation was recognized by Western Blot and immunohistochemistry in clear cell renal cell carcinoma tissues.

The current analysis results reported significant elevated LDL-c and lowered HDL-c concentrations in the patients' group compared to controls. There was a significant positive correlation between serum HDL-c levels and *ATP5G1* expression, while the LDL-c levels negatively correlated with *ATP5G1* expression. Additionally, the logistic regression analysis has shown that *ATP5G1* relative expression in PBMCs and serum HDL-c levels were independent risk factors for schizophrenia.

This finding agrees with Mensi et al., who stated significantly increased serum concentrations of LDL-c in patients with schizophrenia [34]. Also, decreased HDL-c and the presence of dyslipidemia were predominant in patients with first-episode schizophrenia compared to controls, and the incidence of metabolic syndrome was correlated with higher severity of negative symptoms [35]. The elevated HDL-c level in patients under antipsychotic treatment is associated with improvement in negative symptoms in first-episode psychosis [36]. The ATP synthase action is constrained by different aspects like the membrane lipid environment. The lipoxidation-derived protein and lipoxidation influence the ATP synthase subunits [37]. The oxidative damage of ATP synthase prompts an enhanced mitochondrial bioenergetics abnormality, resulting in mitochondrial dysregulation, cellular dysfunction, and neuronal loss [38].

In further studies, we will aim to study the link between plasma lipids and ATP levels and their effects on schizophrenia with another control group with other disorders such as major depression and psychosis.

5. Conclusion

This study revealed a down-regulation of *ATP5G1* expression in schizophrenia, precisely expression in PBMCs, which was found as an independent risk factor. This might give insight into the role of the *ATP5G1* gene in the pathogenesis of schizophrenia. Further studies are needed to evaluate the role of *ATP5G1* in schizophrenia and their impact on ATP production in these patients.

Authors' contributions

Nesreen G. Elhelbawy (the corresponding author), Nesreen G. Elhelbawy and Eman M. Abd El Gayed performed the laboratory investigations and the molecular analysis beside to selecting the study design. Amany A. Saleh and Sally S. Donia were the major contributors in writing the manuscript. Rania M. Azmy and Mohammed S. Abdelshafy collected the samples and analyzed and interpreted the results. All authors shared in writing and revision of the manuscript and approved the final copy.

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Declaration of competing interest

All authors declare no conflicts of interest.

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