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Analysis of ATPase-6 mtDNA mutations and telomere length in patients with bipolar disorder

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Abstract

This study's objective was to analyze ATPase-6 mtDNA mutations and telomere length in patients with bipolar disorder. 37 patients with bipolar disorder and 48 healthy individuals were included in this research. DNA samples of all patients and healthy individuals were isolated. To identify mtDNA mutations in patients, samples were first amplified by PCR, and then Sanger DNA sequencing was performed. RT-PCR method was used for relative telomere length analysis. T112A (m.8860A>G) mutation was detected in all patients (37/37). In addition, T53I (m.8684 C>T) mutations were detected in three patients (3/37) and L156L (m.8994 G>A) and S176N (m.9053 G>A) mutations in one patient each (1/37). Telomere lengths of patients with bipolar disorder were shorter than healthy individuals (p=0.0046). ROC analyses showed that telomere length has a significant diagnostic value for bipolar patients with 94.6% sensitivity and 45.8% specificity (AUC:0.678, 95% CI: 0.568-0.776, p=0.002). There were not statistically significant differences in telomere lengths according to mutation type (p>0.05). Also, there was no relation difference between ATPase-6 mtDNA mutations and telomere length and clinical demographic data (p>0.05). In conclusion, it was shown that patients with bipolar disorder have shorter telomere lengths than healthy individuals. Telomere length may be used as a diagnostic factor. Furthermore, the high frequency of ATPase-6 mtDNA mutations may be part of the genetic background of bipolar disorder. It would be beneficial to support studies with more extensive patient populations to confirm the results we found.

Introduction

Bipolar disorder is a serious mood disorder related with significant psychosocial and economic morbidity (Smith et al. 2012). Bipolar disorder is a highly inherited mental disorder estimated to affect approximately 50 million people worldwide (O'Connell and Coombes 2021). The roles of monoamines (such as serotonin and dopamine) have been thoroughly researched, and early biological investigations of bipolar illness concentrated on the mechanism of action of antidepressants and antipsychotic medications (Kato 2019). Although the etiology of bipolar disorder remains unclear, hereditary factors have been reported to play an crucial role (Arisoy and Oral 2009).

As regulators of both energy metabolism and cell life and death pathways, mitochondria have a important role in the survival or death of neuronal cells (Scaini et al. 2016). Bipolar disorder's pathogenesis has been linked to mitochondrial malfunction. Bipolar disease is linked to malfunctioning mitochondria, which causes significant metabolic disturbance in glia and neurons with high energy requirements. It is believed that the fundamental factor contributing to chronic oxidative stress in bipolar disorder is a mitochondrial malfunction, including disruption of the electron transport chain (Cyrino et al. 2021). A shift toward anaerobic respiration and lactate generation happens when mitochondrial oxidative phosphorylation is hindered (Jeong et al. 2020). The pathophysiology of bipolar disorders may be influenced by mitochondrial DNA (mtDNA) mutations through changes to intracellular calcium signaling systems (Kato and Kato 2000). Many molecular genetic findings related to mitochondria and endoplasmic reticulum stress signaling in bipolar disorder are common to Parkinson's disease and diabetes mellitus (Kato 2005). Though the evidence for the role of mitochondrial dysfunction is accumulating, the underlying pathophysiology of bipolar disorder is still unknown (Bodenstein et al. 2019).

The length of telomeres, the protecting ends of eukaryotic chromosomes, has been studied as a biomarker for the cumulative impact of environmental exposures and life experiences as well as a risk factor for serious diseases over the past ten years (Lin et al. 2019). Telomere length is a sign of cellular aging since they become shorter with each cell division (Squassina et al. 2017). A risk factor for age-related disease, short telomere length is also linked to reduced hippocampal volumes, age-related cognitive decline, and an increased risk of psychiatric disorders (Palmos et al. 2020). Because of its link to memory function and hippocampus morphology, telomere length variation is considered a heritable indicator of risk for neuropsychiatric diseases (Powell et al. 2018). A cell's shortened telomere length reflects its previous mitosis, its cumulative exposure to inflammation and oxidation, and the presence of the telomere-lengthening enzyme telomerase (Lindqvist et al. 2015). The majority of telomere length studies in psychiatry have been on the major depressive disorder. However, more recent research has demonstrated that people with bipolar disorder had shorter leukocyte telomere lengths (Squassina et al. 2017). It is still unclear what psychological variables and actions are connected to telomere shortening (Birkenæs et al. 2021).

In light of all this information, we aimed to analyze ATPase-6 mitochondrial DNA (mtDNA) mutations and telomere length in Turkish patients with bipolar disorder in the current study. We planned to reveal whether there is a difference between telomere lengths in patients with and without ATPase-6 mtDNA mutation and whether there is a difference between these genetic changes and clinical demographic data.

Material and methods

Collection of bipolar patients and healthy individuals

All samples were collected from individuals who were followed up at Atatürk University Training and Research Hospital, Department of Psychiatry. The clinical research ethics committee at Atatürk University approved this study with the ethics approval number 2022/5-14. The study involved a total of 48 healthy individuals and 37 patients over the age of 18 with a diagnosis of bipolar disorder. Blood samples were collected from each volunteer in a five-cc tube with ethylenediamine tetraacetic acid (EDTA).

A psychiatrist, diagnosed bipolar disorder among the patients. The severity of the psychopathological bipolar disorder symptoms was evaluated using the Clinical Global Impression (CGI) Scale (Guy 1976). The Montgomery-Asberg Depression Rating (MADRS) and the Young Mania Rating Scale (YMRS) (Montgomery, S.A., Åsberg 1979) were also used to evaluate the clinical symptoms of the patients.

CGI Scale: It was developed by Guy and colleagues in 1976 (Guy 1976). The scale includes 3 sub-scales. The score varies between 0 and 18. Higher scores indicate more severe clinical deterioration.

MADRS Scale was developed by Montgomery and Asberg in 1979 (Montgomery, S.A., Åsberg 1979). It has ten items that are completed during the clinical interview. Each item has a severity scale from 0 to 6; higher scores reflect more severe depression symptoms.

YMRS was developed by Young and colleagues in 1978 and is one of the most frequently utilized rating scales to evaluate manic symptoms in patients. The scale has 11 items and is based on the patient's subjective report and the clinician's observation of clinical condition over the previous 48 hours. Higher scores show more severe manic symptoms.

Patients with accompanying mental disorders and chronic physical diseases (hypertension, diabetes, organ failure, cancer, etc.) and pregnant patients were excluded from the study. Healthy volunteers did not have any mental and/or chronic physical illness.

Detection of ATPase-6 mtDNA mutations and in silico analysis

DNA was isolated from 200 microliters of blood using an Ecotech DNA isolation kit from Erzurum, Turkey. Each isolation step was carried out in accordance with the recommendation of the relevant company (Dirican et al. 2022). Following DNA isolation, the purity and concentration of DNA samples were assessed at 260/280 nm wavelength using a Take3 Plate (BioTek) spectrophotometry equipment. All DNA samples were kept at -20°C.

Using a SenseQuest Labcycler thermalcycler (Germany), PCR was carried out to examine the ATPase-6 mtDNA mutations. The PCR reaction requires 25 µl of EcoTaq 2× PCR Master mix, 2 µl of forward and reverse primer (10 µM), 10 pg–500 µg template DNA and over with dH_2O. The PCR program was adjusted according to the recommendation of the respective kit manufacturer (Dirican et al. 2022). Primers for the ATPase-6 gene were obtained from an earlier investigation (Avcilar et al. 2016). Following the PCR process, the desired 675 bp bands were verified using a 50 bp marker and ran on a 2% agarose gel with 3 µl of ethidium bromide.

PCR purification using ExoSAP and Sanger DNA sequence analysis with ABI PRISM 310 was carried out (Applied Biosystems; Thermo Fisher Scientific, Inc., Waltham, MA, USA). Unipro UGENE v43.0 tool was used to demonstrate the findings of Sanger DNA sequencing.

The databases FatHMMW, PolyPhen 2, PANTHER, SIFT, PROVEAN, Mutation Assessor, SNAP, and CADD were used for *in silico* study of ATPase-6 mtDNA mutations.

Relative telomere length analysis

Bio-Rad RT-PCR system was used to measure relative telomere length. According to the manufacturer's instructions, 10 µl of iTaq™ Universal SYBR® Green Supermix (2x) (Bio-Rad), 2.5 µl of DNA (25-30 ng), 7.1 µl of dH2O, 0.2 µl of primers were used for each reaction. The 36B4 reference gene was used for normalization. In addition, the RT-PCR program was adjusted according to the recommendations of the relevant company. T/S value was the unit of measurement for RT-PCR. For RT-PCR, previously used primers (36B4 and Telo) were preferred (Gil and Coetzer 2004). The $2^{-\Delta\Delta ct}$ method was used for assesment of telomere length analysis.

Statistical analyzes

GraphPad Prism Version 9.4.0 and MedCalc 18.11.6 programs were used for statistical analysis of all results. Kolmogorov-Smirnov (K-S) test was used for normalization. Comparison of age distribution was performed by Mann-Whitney *U* test. Comparison of gender distribution was performed by χ^2 test. The Mann-Whitney *U* test was used for the difference in telomere length between patients with bipolar disorder and healthy individuals. ROC analysis was performed to reveal the importance of telomere length in diagnosing bipolar patients. The age, smoking status, and telomere length of bipolar disorder patients were also compared using the Mann-Whitney *U* test. The telomere lengths of people with the T112A (m.8860 A>G) and T53I (m.8684 C>T) mutations were compared using the Mann-Whitney *U* test. Statistical significance was defined as a p-value of less than 0.05.

Results

Bipolar disorder patients' and healthy people's characteristics

The characteristics of patients with bipolar disorder and healthy volunteers are summarized in **Table 1**. Thirty-seven patients with bipolar disorder were 21 male and 16 were female. The mean age and standard deviation of the patients were 36.11±11.20. The mean body mass index (BMI) and standard deviation of the patients were 25.73±40.10. Twenty-four of the patients had a smoking habit. Two of the patients had a habit of using alcohol. Three of the patients had the use of drugs. Eight of the patients had a history of suicide attempts. Fifteen of the patients had a family history of psychiatric disorders. The educational status of the patients are mostly primary and secondary graduates (43.24%). The mean and standard deviations of the CGI, YMRS and MADRS disease severity assessment scales at the hospitalization period were 5.13±0.87, 29.72±15.32 and 23.17±14.60, respectively.

The mean age and standard deviation of healthy volunteers were 35.81±10.17. Of the healthy volunteers, 19 were female and 29 were male.

Distribution of ATPase-6 mtDNA mutations and in silico analysis results of patients with bipolar disorder

PCR amplified DNA samples of patients with bipolar disorder, and then they were run in gel electrophoresis using ethidium bromide in 2% gel and visualized in UV. Targeted 675 bp bands were captured **(Figure 1)**. ATPase-6 mtDNA mutations were identified by Sanger DNA sequencing **(Figure 2)**.

All patients with bipolar disorder had the m.8860 A>G mutation (37/37). m.8684 C>T mutation was found in 3 of the patients, and m.8994 G>A and m.9053 G>A mutations were found in 1 each of the patients. Two different mutations were detected in 3 patients and 3 different mutations were detected in 2 patients.

A score called the Grantham value defines the difference in side chain atomic composition, polarity and volume between two amino acids (Grantham 1974; Dirican et al. 2022). The Grantham score is evaluated as conserved (0-50), moderately conserved (51-100), moderately radical (101-150), or radical (≥151) (Abkevich 2004). Among the mutation types defined in bipolar disorder patients in this study, those carrying the T153IA (m.8684 C>T) ATPase-6 mtDNA mutation had the highest Grantham score and this value was determined as 89. The Grantham value was 58 for the T112A (m.8860 A>G) and 46 for S176N (m.9053 G>A) ATPase-6 mtDNA mutation **(Table 2)**.

In this study, eight different *in silico* analysis databases were used for in silico analysis of ATPase-6 mtDNA mutations. According to *in silico* analysis results, it has been reported that m.8860 A>G, m.8684 C>T and m.9053 G>A mutations have benign, pathogenic, or harmful effects in some diseases **(Table 3)**.

Telomere lengths of patients with bipolar disorder compared to healthy volunteers

According to the results obtained, patients with bipolar disorder had statistically significantly shorter telomere lengths compared to healthy volunteers (p=0.0046) **(Figure 3)**. Also, ROC analyzes showed that telomere length has a significant diagnostic value for bipolar patients with 94.6% sensitivity and 45.8% specificity (AUC:0.678, 95% CI: 0.568-0.776, p=0.002) **(Figure 4)**. However, no significant difference could be determined between telomere lengths according to the characteristics of the patients, such as smoking, age, and gender (p>0.05).

Telomere length of individuals carrying ATPase-6 mtDNA mutations

Individuals carrying ATPase-6 mtDNA mutations differed in telomere lengths **(Figure 5)**. When the telomere lengths of individuals having the T112A (m8860 A>G) and T53I (m.8684 C>T) mutations with the highest frequencies were compared, the telomere length of T112A was shorter. However, no statistically significant difference could be determined (p=0.0948) **(Figure 6)**.

Discussion

A dangerous mental illness called bipolar disorder is characterized by manic, depressive and mixed episodes (Müller and Leweke 2016). About 1–2% of the population is afflicted by the disease (Müller and Leweke 2016). Bipolar disorder frequently results in persistent symptoms, issues with psychosocial functioning, cognitive decline, and low quality of life (Bonnín et al. 2019). During growth and development, telomere length is also influenced by genetic and environmental variables (Srinivas et al. 2020). Mood disorders and the effects of mood stabilizers are related to mtDNA abnormalities and/or polymorphisms, altered phospholipid metabolism, glycolytic shift, decreased ATP synthesis, increased oxidative stress, and changes in intracellular calcium (Cikankova et al. 2017). Since mitochondrial dysfunction was initially proposed in the brains of patients with these conditions, variations in mtDNA and nuclear genes encoding mitochondrial proteins have been investigated in bipolar disorder, depression, and other psychiatric disorders (Kasahara and Kato 2018). Therefore, this study analyzed telomere length and ATPase-6 mtDNA mutations in Turkish patients with bipolar disorder.

According to recent studies, mtDNA variations may increase the incidence of diseases such as bipolar disorders, schizophrenia and major depressive disorder (Anglin et al. 2012; Hudson et al. 2014). mtDNA and nuclear genome co-evolution anomalies result in mitochondrial degeneration and an increased risk of bipolar disorder, in addition to environmental influences (Gonzalez 2021). In bipolar disorder and schizophrenia, mtDNA alterations affect neurotransmitter and metabolic processes (Dager et al. 2004). Detected mtDNA mutations have been associated with bipolar disorder and some disease symptoms (Munakata et al. 2004). Genom-wide search done by Ryu et al. (2017) demonstrated mitochondrial single-nucleotide polymorphism and its effects on BD risk (Ryu et al. 2017). B Kato et al. (2021) showed the presence of multiple mtDNA mutations in patients with bipolar disorder. They suggested that among mutations, mutations pertaining to ATPase-6 and 8 may alter the efficiency of ATP synthesis (Kato et al. 2001). Despite the studies above, there have not been many studies of ATPase-6 mtDNA mutations in patients with bipolar disorder. In present study, m.8860 A > G ATPase-6 mtDNA mutation was detected in all patients with bipolar disorder. In addition, m.8684 C > T mutations were found in 3 patients, m.8994 G > A and m.9053 G > A mutations in 1 patient each. According to Kazuno et al. (2009), while the 12358A > G was found to be strongly related to bipolar disorder, the 5460G > A was not (Kazuno et al. 2009). Bipolar disorder was not linked to the mtDNA5460A polymorphism, which decreased intracellular calcium responsiveness (Kazuno et al. 2009). In bipolar disorder patients, Sequeira et al. (2015) discovered the MT-ATP6 mutations m.8794, m.8843, m.9055, and m.9160 (Sequeira et al. 2015).

The difference in side chain atomic composition, polarity, and volume between two amino acids is measured using a score called the Grantham Difference (Grantham 1974). The T153IA (m.8684 C > T) ATPase-6 mtDNA mutation had the highest Grantham score, with 89, among the mutation types identified in bipolar disorder patients in this investigation. The ATPase-6 mtDNA mutations T112A (m.8860 A > G) and S176N (m.9053 G > A) have Grantham values of 58 and 46, respectively.

For the in silico analysis of ATPase-6 mtDNA mutations in this investigation, eight distinct in silico analysis databases were employed. It has been reported that m.8860 A > G, m.8684 C > T, and m.9053 G > A mutations have benign, pathogenic, or damaging consequences in several disorders following the results of in silico investigation.

Compared to healthy volunteers, bipolar disorder patients' telomere length was considerably shorter in this study. Barbé-Tuana et al. (2016) reported that telomere length was shorter in both the early and late subgroups of bipolar disorder subjects compared to the respective controls (Barbé-Tuana et al. 2016). Another study revealed that patients with late-stage bipolar disorder had significantly shorter LTL than those with early-stage bipolar disorder (Huang et al. 2018). According to Lima et al. (2015), bipolar illness patients had shorter telomeres than healthy controls. However, the telomere length of various bipolar disorder subtypes did not significantly differ from one another (Lima et al. 2015). According to Köse Çinar (2017), late-stage patients have shorter TL than early-stage patients and controls (Köse Çinar 2017). Spano et al. showed that the relatively short telomere length in bipolar patients might be an essential criterion to distinguish patients from healthy individuals (AUC; 0.904) (Spano et al. 2022a). When we look at these studies, as in our study, telomere length was found to be short in patients with bipolar disorder. According to ROC analyses, telomere length has a considerable diagnostic value for bipolar patients, with 94.6% sensitivity and 45.8% specificity. Despite this, no discernible difference between telomere lengths according to patient characteristics like smoking, age, and gender could be found. Telomere lengths and the

acceleration of epigenetic aging were shown to be significantly correlated (Fries et al. 2020). According to Spano et al. (2022), telomere length was related considerably to older age (Spano et al. 2022b).

No studies looked at ATPase-6 mtDNA mutations and telomere length together in patients with bipolar disorder. Therefore, this is the first research to examine these two mechanisms in patients with bipolar disorder. Telomere lengths varied amongst patients who had mutations in the ATPase-6 mtDNA. Comparing the telomere lengths of those with the highest frequency of the mutations T112A (m8860 A > G) and T53I (m.8684 C > T), the telomere length of T112A was shorter. No statistically significant difference, however, could be seen.

Limitations of the Study

This study had some limitations. The first one was made without any project support. Second, ATPase-6 mtDNA mutations were screened and telomere length analysis was performed in a small number of patients with bipolar disorder.

Conclusion

In conclusion, this is the first study to analyze ATPase-6 mtDNA mutations and telomere length in Turkish patients with bipolar disorder. The frequency of ATPase-6 mtDNA mutations was found to be high. Telomere length was shown to be shorter in patients with bipolar disorder than in healthy individuals. But, no statistical relation difference was found between ATPase-6 mtDNA mutations and telomere length. In addition, no relationship was found between telomere length and the psychiatric and demographic data of the patients. We believe that looking at these two genetic mechanisms in the larger patient population with bipolar disorder in the future may be valuable in diagnosis or treatment.

Declarations

Ethics statement

The clinical research ethics committee at Atatürk University granted approval for this study with the ethics approval number 2022/5-14.

Data availability statement

The datasets used and analysed during the current study are available

from the corresponding author on reasonable request

Funds Collection: n/a.

Declaration of competing interest

The author declares no conflict of interests.

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Tables

Table 1. Distribution of demographic characteristics of bipolar disorder patients and healthy individuals			
Parameter	**Bipolar (n=37) n (%)**	**Healthy (n=48) n (%)**	***p**
Age (mean±SD, years)	36.11±11.20	35.81±10.17	0.7205[#]
BMI	25.73±40.10	-	-
Disease onset age (mean±SD, years)	22.14±6.12	-	-
CGI scale (Hospitalization period) (mean±SD)	5.13±0.87	-	-
YMRS scale (Hospitalization period) (mean±SD)	29.72±15.32	-	-
MADRS scale (Hospitalization period) (mean±SD)	23.17±14.60	-	-
Gender			
female	16 (43.24)	19 (39.58)	0.7339[##]
male	21 (56.76)	29 (60.42)	
Narcotic drug use status			
yes	3 (8.11)	-	-
no	34 (91.89)	-	-
Alcohol use status			
yes	2 (5.41)	-	-
no	35 (94.59)	-	
Smoking status			
drinks	24 (64.86)	-	-
not drinking	13 (35.14)	-	-
Suicide attempt status			
yes	8 (21.62)	-	-
no	29 (78.38)		
Age at diagnosis			

(mean±SD, years)	25.47±7.85	-	-
Individual with a family history of psychiatric illness			
yes			
no	15 (40.54)	-	-
	22 (59.46)	-	-
Number of hospitalizations (mean±SD)	6.31±5.25	-	-
Educational status			
Primary and secondary school	16 (43.24)	-	-
High school	12 (32.43)	-	-
University	2 (5.41)	-	-
can't read	7 (18.92)	-	-

*p<0.05 was considered statistically significant. # Mann Whitney U test and ## χ^2 test were used. CGI: Clinical Global Impression Scale, YMRS: The Young Mania Rating Scale, MADRS: Montgomery-Asberg Depression Rating, BMI: Body mass index

Table 2. ATPase-6 mtDNA mutations detected in patients with bipolar disorder

Gene	Nucleoid Position	Nucleotide Exchange	Amino Acid Change	Mutation Type	Grantham Value	Mutation Rate (Number Of Mutations/ Total Number Of Patients)
ATPase-6	8860	AàG	T112A	Missense	58	37/37
ATPase-6	8684	CàT	T53I	Missense	89	3/37
ATPase-6	8994	GàA	L156L	Transition	-	1/37
ATPase-6	9053	GàA	S176N	Missense	46	1/37
Total number of mutations detected						**42**
Presence of 1 mutations in a patient						**33**
Presence of 2 mutations in a patient						**3**
Presence of 3 mutations in a patient						**1**

Table 3. In silico analysis results of ATPase-6 gene variants

Gene	Nucleoid Position	Allele	FatHMMW	Polyphen2	PANTHER	SIFT	PROVEAN	Mutation Assessor	SNAP	CADD
ATPase-6	8860	A>G	Neutral	Benign	Neutral	Neutral	Deleterious	Medium impact	Disease	Neutral
ATPase-6	8684	C>T	Neutral	Benign	Neutral	Neutral	Neutral	Neutral impact	Neutral	Neutral
ATPase-6	9053	G>A	Neutral	Benign	Neutral	Neutral	Neutral	Low impact	Neutral	Neutral

FatHMMW : http://fathmm.biocompute.org.uk/

Polyphen2: http://genetics.bwh.harvard.edu/pph2/

PANTHER: http://pantherdb.org/

SIFT : https://sift.bii.a-star.edu.sg/

PROVEAN : https://www.jcvi.org/research/provean

Mutation Assessor: http://mutationassessor.org/r3/

SNAP : https://bromberglab.org/project/snap/

CADD : https://cadd.gs.washington.edu/

Figures

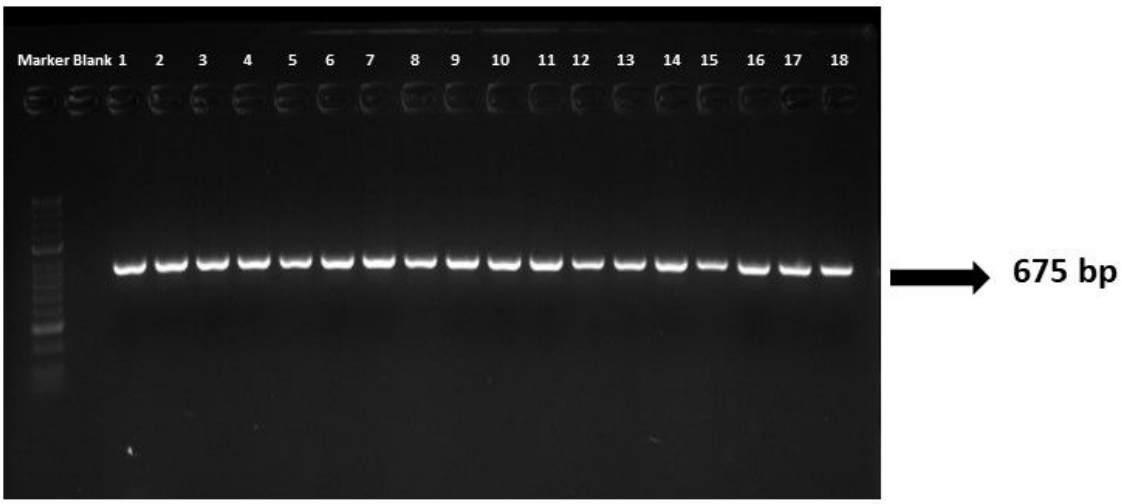


Figure 1

Image of bands in gel electrophoresis after PCR of 675 bp ATPase-6 gene. (1-18: samples; A 50 bp marker was used.)

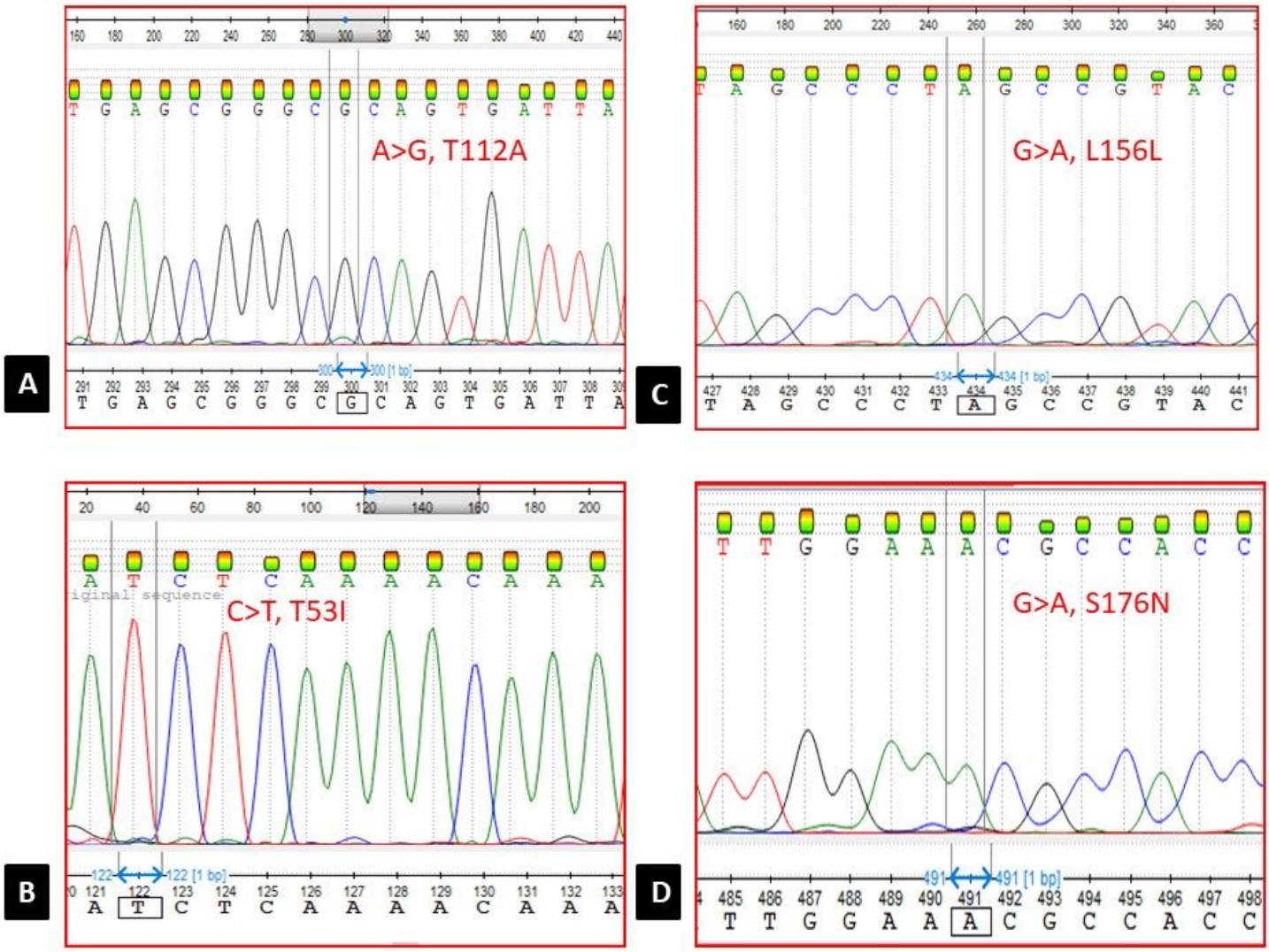


Figure 2

Sanger DNA sequence analysis results of ATPase-6 mtDNA mutations. A) m.8860 A>G (T112A), B) m.8684 C>T (T53I), C) m.8994 G>A (L156L), D) m.9053 G>A (S176N)

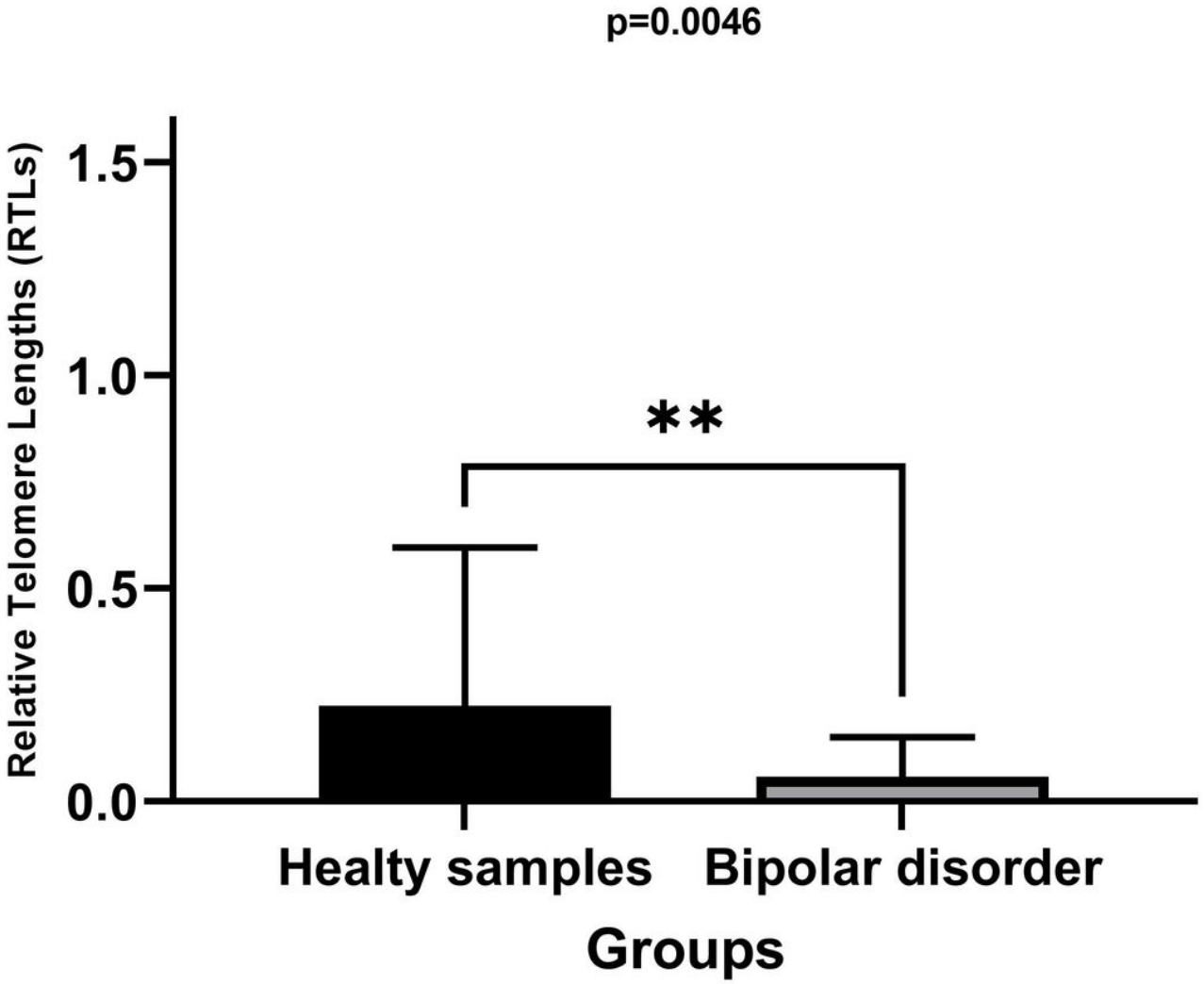


Figure 3

Comparison of relative telomere length (RTLs) in patients with bipolar disorder and healthy individuals

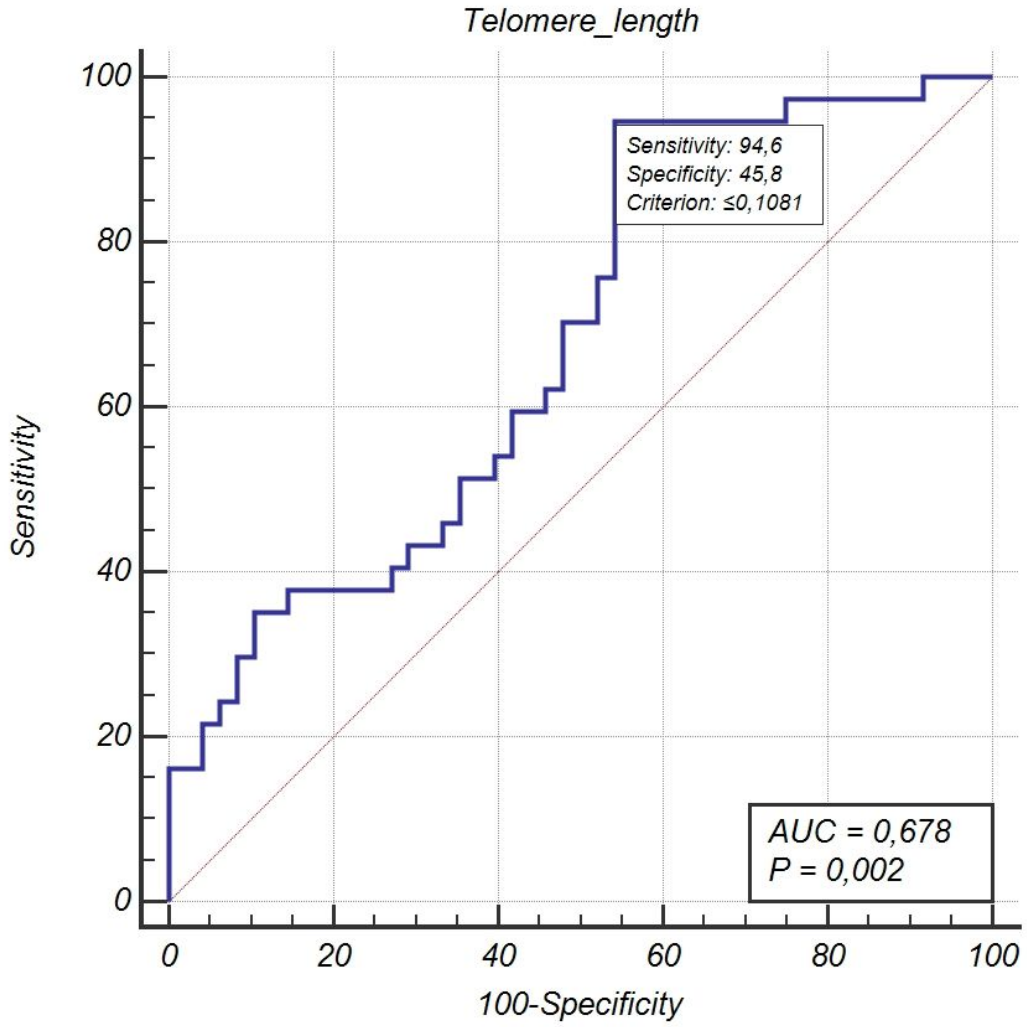


Figure 4

ROC analysis result of telomere length. (94.6% sensitivity, 45.8% specificity, AUC:0.678, 95% CI: 0.568-0.776, p=0.002)

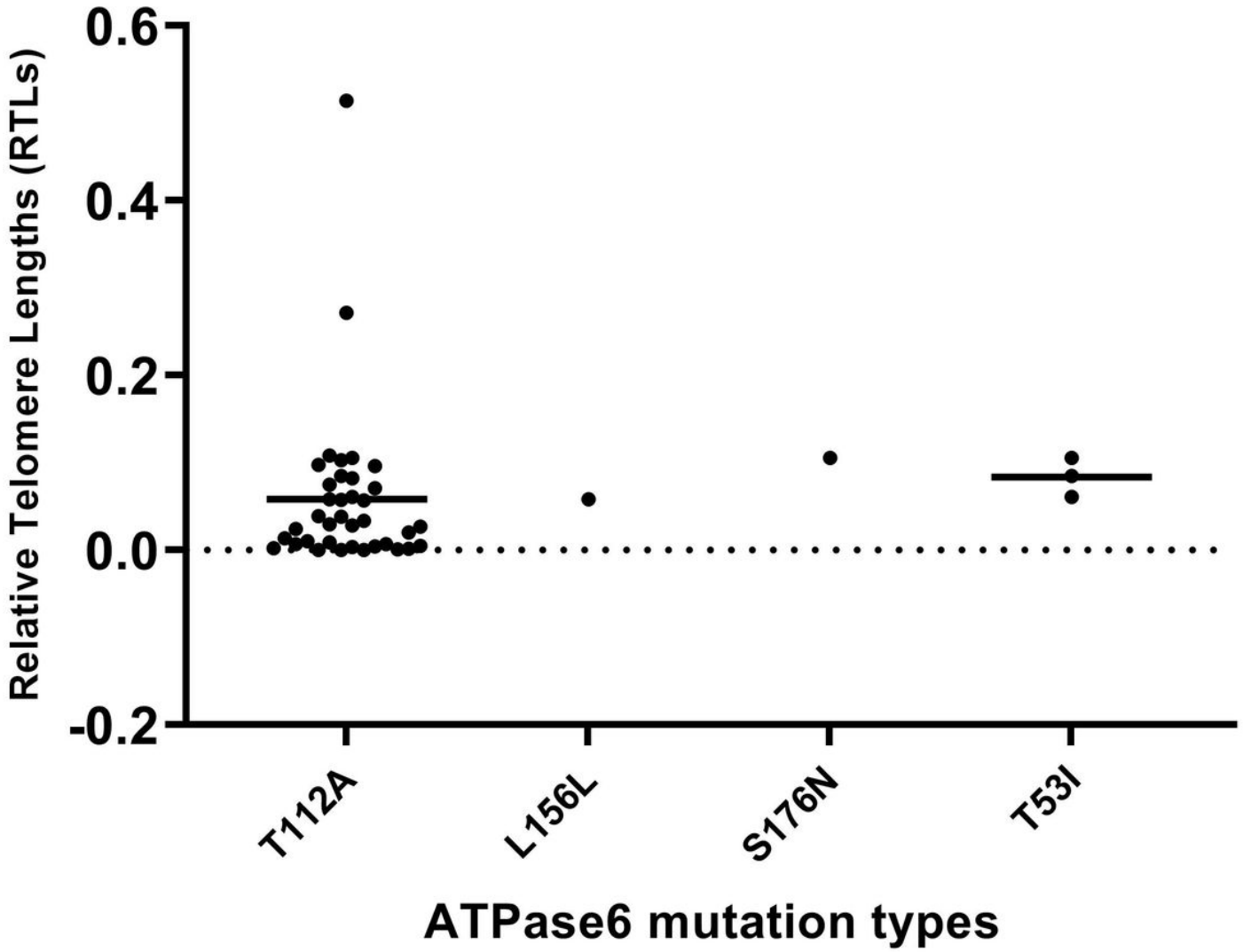


Figure 5

Distribution of telomere length by mutation type in patients with bipolar disorder carrying ATPase-6 mtDNA mutation

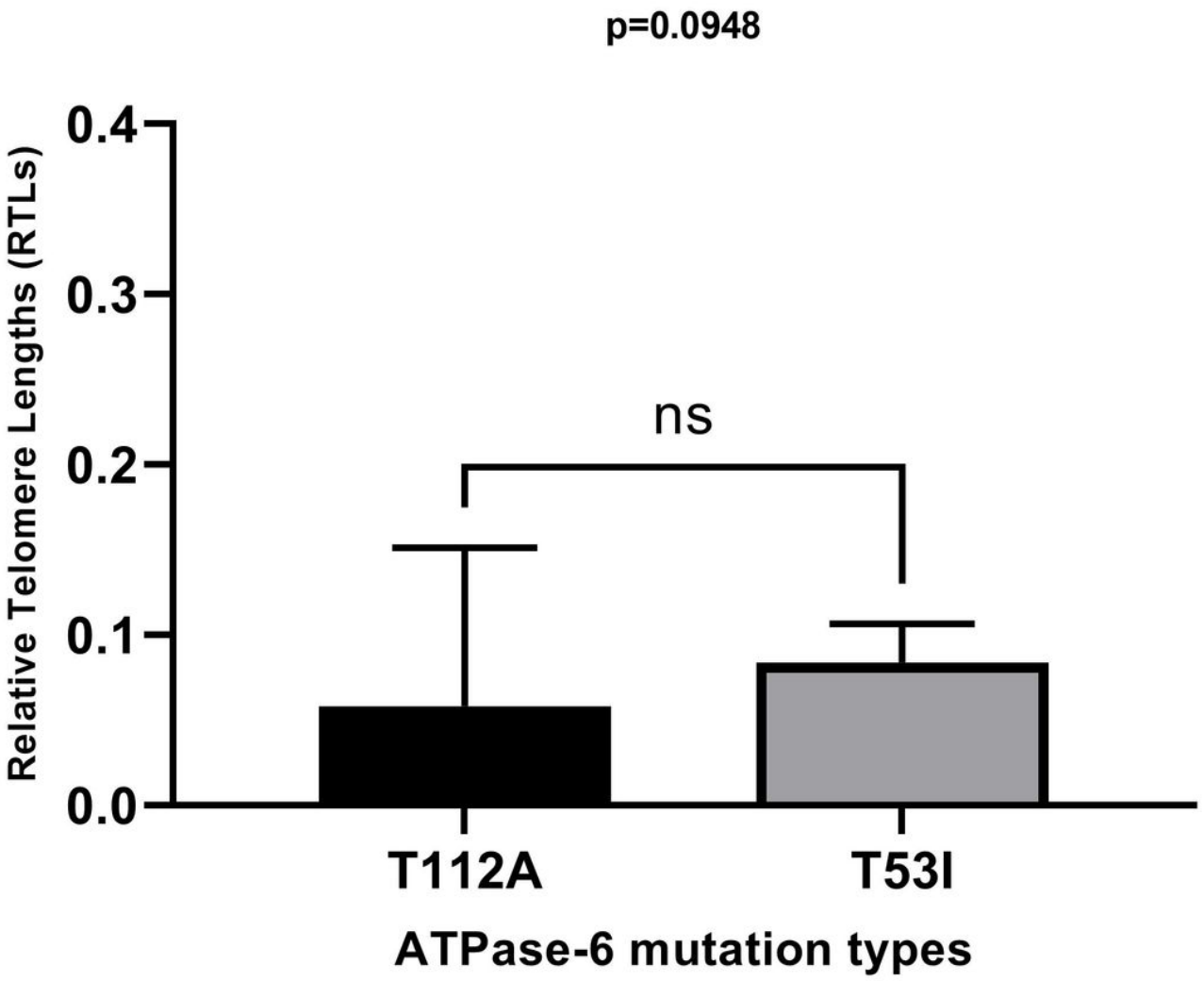


Figure 6

Distribution of telomere length in patients with bipolar disorder according to the highest frequency m.8860 A>G (T112A) and m.8684 C>T (T53I) ATPase-6 mtDNA mutations