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Propranolol decreases *DRD3* and *SLC1A2* gene expression in patients with essential tremor

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ABSTRACT

BACKGROUND

Essential tremor (ET) is the most common disease among movement disorders. Genes such as essential tremor 1-4 (*ETM* 1-4), *HS1*-binding protein-3 (*HS1BP3*), dopamine receptor *D3* (*DRD3*), leucine-rich repeat and Ig domain containing 1 (*LINGO1*), glial high affinity glutamate transporter member 2 (*SLC1A2*), *FUS*, high temperature requirement A2 (*HTRA2*) and *TENM4* had been shown to be responsible for the genetic inheritance of the disease. The aim of the present study was to investigate the effect of propranolol on the expression of *DRD3*, *SLC1A2*, and *HTRA2* genes in patients with ET.

METHODS

A study of non-randomized experimental design was conducted involving 76 subjects. They were divided into two groups: 38 patients with ET in the patient group (Group 1) and 38 healthy people in the control group (Group 2). *DRD3*, *SLC1A2* and *HTRA2* gene expressions were assessed before and after 8 weeks of propranolol treatment. Fahn-Tolosa-Marin tremor scale results were compared before and after propranolol administration. Kruskal Wallis test was used to determine differences in gene expressions between the groups.

RESULTS

D3 dopamine receptor and *SLC1A2* gene expression in the patient group appeared to be lower than in the control group ($p < 0.001$). However, the *HTRA2* gene expression level was significantly higher in the patient group ($p < 0.001$).

CONCLUSION

D3 dopamine receptor and *SLC1A2* gene expressions were decreased in ET patients which at first glance can be explained in relation to etiology, but after the treatment it was not increased as expected but decreased even more.

Key words: Essential tremor, *DRD3*, gene expression, *HTRA2*, propranolol, *SLC1A2*

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INTRODUCTION

Essential tremor (ET) is the most common movement disorder with motor and non-motor symptoms. Although the disorder can occur at any age, the incidence increases with age.^(1, 2) Positive family history in individuals with ET varied between 17.4% and 100%.⁽²⁾ Most of the patients with ET were not diagnosed correctly and had missed the appropriate treatment options. The pathophysiology of the disease is still unknown. The clinical presentation of ET was the 6-12 Hz kinetic and postural tremor, which occurred in the hands and arms by the voluntary movements. Other anatomical parts affected by tremors were head, vocal cords, trunk, legs and face regions. Essential tremor is currently revealed as a heterogeneous disease.^(3, 4)

The etiology of ET is mostly unexplained. About half of the cases of ET appear to result from a genetic mutation, although a specific gene has not been identified. This form is referred to as familial tremor and is an autosomal dominant disorder. The variability in age of onset, the presence of sporadic cases, and incomplete concordance of ET among monozygotic twins suggest that environmental factors play a role.^(5- 8) The relationship of the disease to non-motor symptoms of such entities as parkinsonism, dystonia, cerebellar dysfunction, sleep disturbances, mild cognitive impairment, and cognitive disorder, personality and temperament disorder, showed that the disease included not only the motor but also non-motor dysfunction.^(9- 11) Essential tremor is autosomal dominantly inherited with an incomplete penetration. However, some families may also have polygenic inheritance.⁽¹²⁾ A study at Yale University found that ET patients and their families knew the most basic, observable features of ET (e.g., the presence of hand tremors). However, 16.7% of the affected did not endorse genes as a cause for ET.⁽¹³⁾

Treatment of patients with ET can be divided into two parts, i.e. medical and surgical parts. Pharmacological agents such as propranolol,

pirimidone, topiramate, and gabapentin are preferred as a medical treatment, whereas surgical treatment may include thalamotomy, lesion surgery, or deep brain stimulation.^(14, 15) A randomized controlled trial showed that propranolol reduced tremor amplitudes as measured by accelerometry by a mean of 55%.⁽¹⁴⁾

The dopamine receptor D3 (*DRD3*) gene is localized on chromosome 3q13.31 and has been shown to be associated with ET together with the *ETM1* locus. The D3 receptors are primarily expressed in mesolimbic areas associated with cognitive functions and motivated behaviour and have been implicated in the antipsychotic effects of neuroleptics as well as in the conditioning to psychostimulants.⁽¹⁶⁾

The glial high affinity glutamate transporter member 2 (*SLC1A2*) gene as a member of the glutamate transport system, also known as glutamate transporter 1, contains five different members with variable expression patterns in the central nervous system. Due to the physiological and pathological effects of possible mutations in the solute carrier family 1 member 2 (*SLC1A2*) gene, these mutations were assumed to cause glutamatergic defects in the central nervous system of ET patients. Although the *SLC1A2* expression was shown in peripheral organs, the SymAtlas results showed that the *SLC1A2* gene is mostly expressed in the brain. This astroglial specific high-affinity glutamate transporter has been reported for expression alterations in psychiatric disorders.^(17,18)

The high-temperature-regulated A2 (*HTRA2*) gene is a serine protease that is released during apoptosis from the mitochondria to the cytosol and plays role in many neurodegenerative diseases.⁽¹⁹⁻²¹⁾ High-temperature-regulated A2 has already been designated as *PARK13* which may cause Parkinson's disease, although there are still discrepancies among the study results.⁽²²⁾

A case control study involving 45 subjects with ET and 13 subjects without tremor showed that the genotype frequencies did not differ significantly between subjects with ET and healthy controls.⁽²³⁾

To the best of our knowledge this is the first study aiming to present the expression change in *DRD3*, *SLC1A2*, and *HTRA2* genes before and after the treatment of ET patients with propranolol.

METHODS

Research design

This non-randomized experimental study was carried out in the Departments of Medical Genetics and Neurology, Faculty of Medicine, Erciyes University between July 2016 and January 2019.

Research subjects

A total of 76 subjects with the diagnosis of ET and healthy subjects were recruited. They were stratified into the ET group and the control group consisting of the healthy subjects. The sample sizes were determined using effect size =0.6; $\alpha=0.05$ and $\beta=0.2$, resulting in 38 subjects for each group. The inclusion criteria of the ET patients presenting with an involuntary rhythmic sinusoidal oscillation of a body part without any known causes, were age between 18 and 60 years, having no additional disease and being only on propranolol treatment at 40 mg twice per day for 8 weeks.

Exclusion criteria were hyperthyroidism, psychiatric disorders such as depression, anxiety, psychosis, etc., cerebrovascular disease, diabetes mellitus, other forms of tremor in the patient including neuropathic tremor, dystonic tremor, orthostatic tremor, etc. and being on treatment that increases tremor severity (valproic acid, etc.). The study included 38 patients with ET and 38 healthy individuals as a control group. The control group included healthy people with the age of 18-60, chosen from the epidemiological area of the EUMF hospital, who had no other neurological disorder or chronic medical disease as a result of the evaluation done by an experienced neurologist. Patients admitted to the neurological movement disorders outpatient clinic of Erciyes University, Faculty

of Medicine and diagnosed with ET by an experienced neurologist were included into the study using the Fahn-Tolosa-Marin Clinical Rating Scale for Tremor scale (FTM).⁽²⁴⁾ Tremor severity was given a score between 0 and 4 in FTM. In order to determine the tremor severity-frequency of the patients with the FTM, the device was given to patients to measure the tremor frequency with both hands and they were asked to hold for 10 sec.

Primer sequences used for gene expression

The RNA was isolated by leukocyte isolation using Red Cell Lysis solution (Sigma-Aldrich, St. Louis, Missouri, United States) and TRIzol, a chemical solution used in the extraction of DNA, RNA, and proteins from cells (Invitrogen, Thermo Fisher Scientific, California, United States). Quantitative real-time PCR which is a PCR-based technique that compares amplification of a target DNA sequence with quantification of the concentration of the selected reference (housekeeping) DNA were performed on Light Cycler 480 II (Roche Diagnostics Ltd., Rotkreuz, Switzerland). The expression levels of *DRD3*, *SLC1A2* and *HTRA2* genes were measured via specific Real Time ready Catalogue primary-probe kits (Roche Diagnostic GmbH, Mannheim, Germany) (**Table 1**). The β -Actin gene was used as the reference housekeeping gene. Data were analyzed via the Light Cycler 480 Software (version 1.5.0 SP4) (Roche Diagnostics Ltd., Rotkreuz, Switzerland) using the 'delta-delta Ct' formula to calculate the relative fold gene expression. Gene expression results were compared between control, pre- and post-treatment groups.

Statistical analysis

Statistical analysis was performed using GraphPad Prism 6.0 (GraphPad Software, CA, USA). The histogram, Quantile-Quantile Plot and Shapiro-Wilks tests were used to evaluate the normal distribution of the data and an independent-test was used. In the case of an abnormal data

Table 1. Primer sequences used in expression

Gene	Assay ID	Primer sequence (5'-3')
<i>SLC1A2</i>	111792	F: CCTGCCAACAGAGGACATC R: GACTGAAGTTCTCATCCTGTCCA
<i>DRD3</i>	113554	F: TTCGGGAGAAGAAGGCAAC R: GCAGCCAGCAGACAATGA
<i>HTRA2</i>	145466	F: TCAGCATGGTGTACTCATCCA R: AGCTTCATAA

distribution being found, the nonparametric Mann-Whitney test was used to determine the difference between the groups. A Chi square test was used for comparison of distribution regarding gender of participants. $p < 0.05$ was considered as the significance level.

Ethical clearance

Ethical approval (No: 2016/297) was obtained from the Ethical Committee of Erciyes University Medical Faculty.

RESULTS

A total of 76 subjects participated in the study, and were stratified into the ET group and the healthy control group. The ET subjects were given propranolol for 8 weeks (Figure 1).

At base line there was no significant difference in age and gender between the ET and control groups. But the gene expression was significantly different between the ET and control groups. The *DRD3* and *SLC1A2* expressions

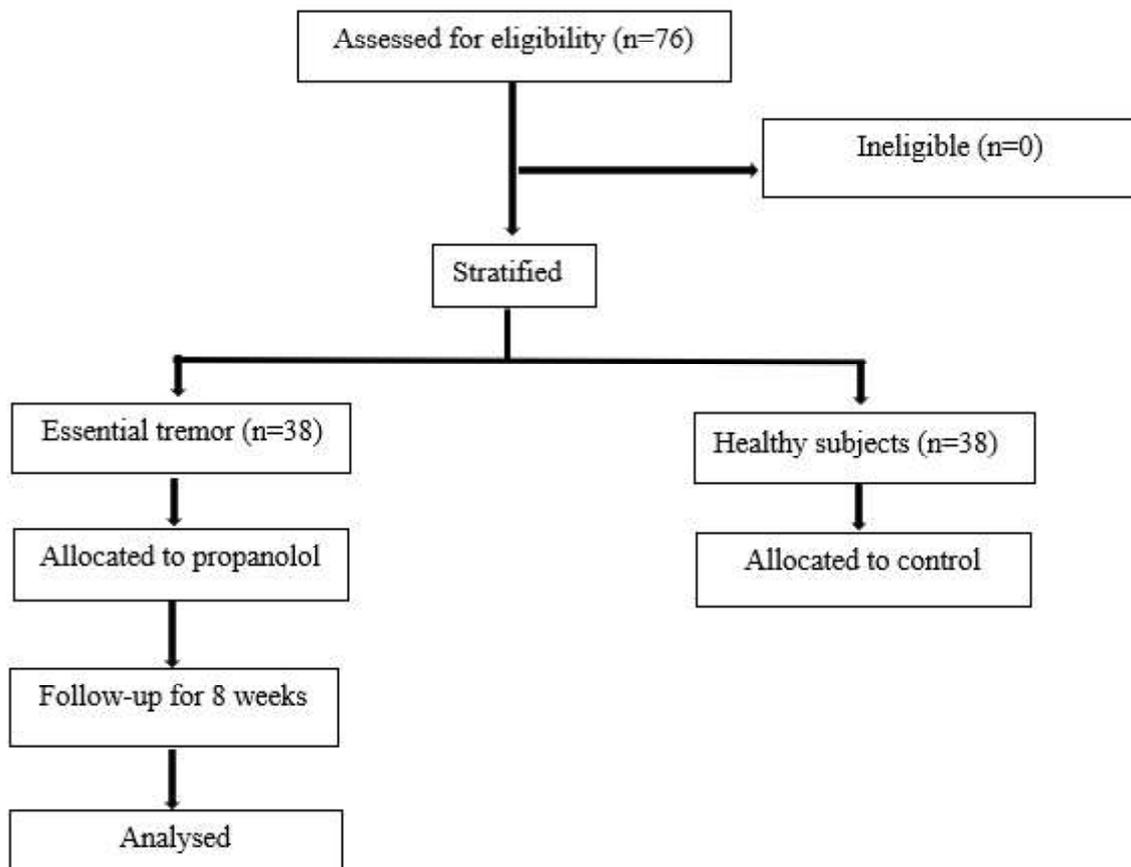


Figure 1. Flow diagram of the participants

Table 2. Distribution of demographic characteristics and FTM-TRS grade within the ET and control groups

Characteristics	Treatment group (n=38)		Control group (n=20)	p value
	Before treatment	After treatment	-	
Age (years)	36.7 ± 12.2		37.35 ± 1.1	>0.05
Gender				
Male	25		12	>0.05
Female	13		8	
FTM-TRS Grade	2.7 ± 1	2.1 ± 0.8	-	<0.0001

were significantly lower in the ET group in comparison to the healthy group ($p < 0.001$), but the *HTRA2* gene was significantly higher in the ET group ($p < 0.001$) (Table 2).

Pre- and post-treatment expression levels of the *DRD3*, *SLCIA2* and *HTRA2* genes were compared in the ET group. Propranolol significantly decreased *SLCIA2* expression, but the decrease in *HTRA2* and *DRD3* gene expressions were not significant.

We did not compare gene expression after 8 weeks between the ET group and the control group, as the latter did not receive any treatment.

DISCUSSION

Our results showed reduced *DRD3* and *SLCIA2* gene expression and increased *HTRA2* gene expression levels in the patient group. The *DRD3* receptor is a protein consisting of 446 amino acids. ⁽²⁵⁾ The *DRD3* receptor is found widely in the brain in the control centers for sensing, affect and motor functions, nucleus accumbens, ventral tegmental area, substantia nigra, limbic region, and basal ganglia. ^(26,27)

In the present study, the expression level of the *DRD3* gene was lower in the patients with

ET than in the control group. Expression of the *DRD3* gene was lower after treatment with propranolol. Since the dopamine level was low in the patients with ET, the low mRNA level might be due to polymorphisms in this gene. ⁽²⁸⁾ A further decrease in *DRD3* expression under the propranolol treatment observed in our study possibly indicates that propranolol does not affect the expression of this gene and that decreased level of mRNA is a feature of ET.

Jiménez-Jiménez et al. ⁽²³⁾ in a study with *DRD3* rs6280, *SLCIA2* rs3794087, and *MAPT* rs1052553 gene variants, which was carried out on 11 Spanish families with ET clinical signs and the risk of these genes for ET inheritance, concluded that no relationship exists between *DRD3* and *SLCIA2* gene variant and ET. Although an increased risk for the *MAPT* variant was detected, this result was not confirmed by a family-based study.

SLCIA2, which encodes the glutamate transporter and exhibits high affinity especially in astroglial cells, is a brain-specific gene. ⁽²³⁾ The present study found that the expression level of the *SLCIA2* gene was lower in the patient group. The expression level of the *SLCIA2* gene was found to be even lower in the post-propranolol

Table 3. Gene expression comparison between the treatment (before and after 8 weeks propranolol treatment) and control groups

	ET diagnosed patient group		Control group	p value
	Before treatment	After treatment		
<i>DRD3</i>	1.2 ± 1.6	0.7 ± 0.6	5.3 ± 4.7	<0.0001
<i>SLCIA2</i>	2.3 ± 3	0.4 ± 4	10.6 ± 8.2	<0.0001
<i>HTRA2</i>	6.2 ± 7.5	4.3 ± 6	1.8 ± 0.98	>0.05

treatment period. As the glutamate level is high in ET patients, the low level of *SLC1A2* mRNA, which is known as one of the glutamate carriers, may be due to polymorphisms in this gene.

In another study on exome sequencing done by Gulsuner et al. ⁽²⁹⁾ a rare variant of the ET-associated HtrA serine peptidase 2 gene (*HTRA2*) was described in a family of 6 generations. The *HTRA2* gene encodes a 458 amino acid serine protease found in the intermembrane region of mitochondria. The high-temperature-regulated A2 is known to be effective in the development of many neurodegenerative diseases as well as in apoptosis. ^(30, 31) In the present study, the expression level of the *HTRA2* gene was higher in the patient group. Despite a slight decrease in the post-propranolol treatment period, there was a higher expression in the patient group than in the control group. However, the statistically insignificant difference in *HTRA2* gene expression seen in the present study could be in parallel with the fact that the effect of propranolol on apoptosis has not yet been fully elucidated.

Propranolol is a nonselective beta-adrenergic receptor antagonist. The mechanism of action suggests that it blocks a protein target in the form of peripheral beta-2 receptors. There are different studies in the literature about the effect of propranolol on genes. ⁽³²⁾

The mechanisms underlying the properties of propranolol in gene expression are unclear at present. To explain these mechanisms more comprehensive investigations (*in vivo* and *in vitro*) should be carried out with different doses of propranolol and the protein levels in 3D cultures should be determined in future. Although *HTRA2* expression level was found to be increased in the ET group, the increase was not statistically significant. Therefore further investigations are needed with larger samples to find out whether our results occurred by chance or there actually was a statistically significant increase in gene expression level.

As a limitation of this study, only the sample size can be pointed out. For validation of obtained

results other similar projects with larger samples must be carried out. Also, the effects of different dosages of propranolol could not be compared because of budget limitations.

CONCLUSION

The gene expression study previously performed with propranolol showed that propranolol generally suppresses protein synthesis and gene expression. According to our findings, *DRD3* and *SLC1A2* gene expressions were decreased in ET patients which at first glance can be explained in relation to etiology, but after treatment it was not increased as expected but decreased even more.

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CONFLICT OF INTEREST

There is no conflict of interest between authors.

CONTRIBUTORS

NK conceived the concept and wrote the first draft of the manuscript. MG analyzed the data. MK contributed to the writing of the manuscript. AB, NT, MM jointly developed the structure and arguments for the paper. MD made critical revisions. All authors reviewed and approved the final manuscript. 

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