ABSTRACT

UNIVERSAL MEDICINA

Propranolol significantly reduced DNA polymerase β expression in patients with essential tremor

Nefise Kandemir¹,², Sercan Kenanoglu¹, Murat Gultekin³, Nuriye Gokce¹, Hilal Akalin¹,⁴, Nazife Tascioglu¹, Meral Mirza³, Emel Koseoglu¹, and Munis Dundar¹*

BACKGROUND
Essential tremor (ET) is the most common movement disorder. Propranolol is a first-line medication for ET. We aimed to evaluate the effect of propranolol on the expression of poly (ADP-ribose) polymerase 1 (PARP1) and DNA polymerase beta (POLB) genes, which are known to be related to neurodegenerative diseases, in patients with ET.

METHODS
Thirty-five healthy volunteers and thirty-five patients followed up with essential tremors were included in a non-randomized control experimental study. Expressions of PARP1 and POLB genes were compared between the control group and the patient group. In addition, pre- and post-treatment gene expression levels and Fahn-Tolosa-Marin tremor scale values of the patient group were compared after 8 weeks of propranolol treatment. The Wilcoxon rank and Mann Whitney U tests were used to analyze the data.

RESULTS
At baseline, PARP1 expression was significantly lower in the ET group than in the control group. (p<0.001). POLB gene expression was significantly higher in the pre-treatment ET group than in the controls (p<0.05). There was no significant difference in PARP1 expression levels before and after 8 weeks of propranolol treatment. POLB gene expression was significantly higher in the pre-treatment group than in the post-treatment group (p<0.001).

CONCLUSION
Propranolol significantly decreased POLB gene expression but there was no significant difference in PARP1 gene expression levels in the patient group, after 8 weeks of propranolol treatment.

Keywords: Tremor, genes, propranolol, essential tremor

¹Department of Medical Genetics, Erciyes University Faculty of Medicine, Kayseri, Turkey
²Department of Medical Genetics, Diskapi Yildirim Beyazit Training and Research Hospital, Ankara, Turkey
³Department of Neurology, Erciyes University Faculty of Medicine, Kayseri, Turkey
⁴Department of Medical Genetics, Ahi Evran University Faculty of Medicine, Kirsehir, Turkey

*Correspondence:
Munis Dundar, Professor
Erciyes University Medical School, Department of Medical Genetic, 38039 Kayseri/Turkey
Mobile: +905326486116
E-mail: dundar@erciyes.edu.tr
ORCID ID :0000-0003-0969-4611

Date of first submission, June 21, 2021
Date of final revised submission, September 29, 2021
Date of acceptance, October 1, 2021

This open access article is distributed under a Creative Commons Attribution-Non Commercial-Share Alike 4.0 International License

INTRODUCTION

Essential tremor (ET) that provokes uncontrolled and rhythmic contractions and relaxations (shaking, twitching movements) is one of the progressive neurologic movement disorders. The disease is also called idiopathic tremor or benign tremor. Even though the disease can occur at any period of life, it is more frequently seen in the older age group. The prevalence of ET has a wide range, from 1% in the general population to 5% in the elderly. The main motor dysfunction of ET is a kinetic tremor that occurs during voluntary movements of the hands and arms and over time spreads to the head, vocal cords, jaw, and other anatomical parts. Another common sign of ET is bilateral postural tremor. Essential tremor is a clinically heterogeneous disease and also comprises many disorders such as cognitive disorders, dementia and sensory and psychiatric disorders. Age and family history can be considered as major risk factors for ET. The diagnosis is made based on the typical pattern of tremor and the exclusion of other diseases with tremor. Despite its high prevalence, especially in the elderly, the pathophysiology of the disease is still unclear, although many cases seem to be familial.

There are still several parts in the etiology of ET which need to be clarified but the most known substantial etiological factors are genetic and environmental. Many large family group samples show an autosomal-dominant pattern. First-degree relatives of ET patients have a greater risk of ET and the risk increases if the tremor starts at an early age. Considering that more than half of the affected individuals have a family history, genetics plays a substantial role in the disease. In addition, there is higher concordance in monozygotic twins than in dizygotic twins. Some studies showed that concordance among monozygotic twins is approximately 60% but reported studies about sporadic cases and variable age of onset in some familial cases are arguments for nongenetic factors. A positive family history is quite frequent in patients with ET, among whom a significant number of families presumably have autosomal dominant inheritance, while patients with a positive family history have a younger age of onset for the disease.

Essential tremor and some neurodegenerative diseases such as Parkinson’s disease (PD), dystonia, and progressive supranuclear palsy (PSP) overlap in clinical and pathological features. Some post-mortem examinations have shown that there are tau aggregates in brain samples of patients with ET, similar to those in PSP. Besides, it has been reported that tremors are present also in PD as well as in ET.

There are several reported responsible genes for the inheritance of the disease, for example, HS1-binding protein-3 (HS1BP3), essential tremor 1-4 (ETM1, ETM2, ETM3, ETM4), dopamine receptor D3 (DRD3), glial high-affinity glutamate transporter member 2 (SLC1A2), high-temperature requirement A serine peptidase 2 (HTRA2), fused in sarcoma (FUS), leucine-rich repeat and Ig domain containing nogo receptor-interacting protein 1 (LINGO1), and teneurin transmembrane protein 4 (TENM4).

Patients with ET have medical and surgical treatment options. Propranolol, primidone, topiramate, and gabapentin are generally first choice medicines and another treatment option is surgery that includes thalamotomy, tumoral surgery, and deep brain stimulation.

Poly (ADP-ribose) polymerase 1 (PARP1) is an ADP-ribosylating enzyme essential for initiating various forms of DNA repair. In the pathological condition, extensive DNA damage in cells results in PARP1 cleavage (inactive), preventing DNA repair and thereby leading to cell apoptosis or necrosis.

Poly (ADP-ribose) polymerase 1 is a gene encoding the poly (ADP-ribose) transferase enzyme and is localized in human chromosome 1 (1q42.12). PARP1 encoded by this gene can be activated by DNA damage; it is a nuclear enzyme involved in DNA repair, genomic stability, and many physiological processes such as cell
apoptosis. *PARP1* can act as a sensor for DNA damage. After binding to damaged DNA, *PARP1* binds covalently to nuclear acceptor proteins by forming homodimers and catalyzes the cleavage of NAD + to nicotinamide and ADP-ribose to synthesize long-branching poly (ADP ribose) polymers.\(^{(15)}\)

The DNA polymerase beta (POLB) is a gene localized on the 8th chromosome 8p11.21 region, and is an important type of DNA polymerase that participates in gap-filling DNA synthesis. The protein is normally found in the cytoplasm and functions as a monomer but migrates to the nucleus in case of DNA damage.\(^{(16)}\)

In the literature, many studies observed changes in the expression levels of *PARP1* and *POLB* genes in diseases with pathological features like those of ET. However, no studies have been conducted to evaluate the effect of propranolol on *PARP1* and *POLB* genes in ET patients.

In this study we therefore investigated the effect of propranolol on *PARP1* and *POLB* genes in patients with essential tremors (ET), which are known to be related to neurodegenerative diseases.

**METHODS**

**Research design**

This was a non-randomized control experimental study that was conducted on 35 patients diagnosed with ET in Erciyes University Medical Genetics and Neurology Departments between July 2016 and January 2019.

**Research subjects and intervention**

Thirty-five healthy volunteers and thirty-five patients who were followed up with essential tremors were included in the study after they were assessed by the Erciyes University Adult Neurology and Medical Genetics Departments. Volunteers and patients were assessed by neurological examination and the Fahn-Tolosa-Marin Clinical Rating Scale. As a result, those without neurological disorders or chronic medical diseases were selected as the control group. Power analysis was performed for 35 patients and 35 healthy volunteers to determine sample size. The effect size was 0.082, 1-beta was 0.924 and alpha 0.05.

The inclusion criteria of ET patients were age between 18 and 60 years, diagnosed with ET by a specialist neurologist, having no additional disease and receiving 40 mg propranolol twice a day for eight weeks. Exclusion criteria include patients with hyperthyroidism, diabetes mellitus, psychiatric diseases, tremors (neuropathic, dystonic, and orthostatic), persons receiving treatment that increases tremor and patients under the age of 18.

The control group consisted of volunteers aged between 18 and 60 who were medically healthy, did not have any neurological diagnosis and did not have a chronic disease, as a result of the evaluation made by a specialist neurologist.

**Measurements**

The Fahn-Tolosa-Marin Clinical Rating Scale for Tremor (FTM) was used to assess the essential tremor.\(^{(17)}\) Fahn-Tolosa-Marin is a scale used to evaluate resting, postural and action tremors. There are up to five ratings representing its severity. Tremor severity is evaluated by giving an FTM score between 0 and 4. Briefly, the FTM procedure is as follows: the patients are given a device which they hold for 10 seconds to define the tremor severity and frequency. The degree of the scale is directly proportional to the severity of the disease. FTM was measured only in the patient group before and after propranolol treatment, and the two groups were compared statistically.

**Laboratory analysis**

After peripheral blood samples had been taken from each of the patients and healthy volunteers into 10 ml EDTA tubes, leukocyte isolation was performed in the laboratory of the Erciyes University Medical Genetics Department with the red cell lysis method. Leukocytes taken
in TRIzol™ reagent were stored at -20°C until RNA isolation (Invitrogen, Thermo Fisher Scientific, California, United States). RNA isolation was performed using the phenol-chloroform method. cDNA synthesis was done using the EvoScript Universal cDNA Master Mix kit. Expressions of PARP1 and POLB genes were performed by using the quantitative real-time PCR method on the Light Cycler 480 II device (Roche Diagnostics Ltd., Rotkreuz, Switzerland) using Real-Time ready Catalog primary-probe kits (PARP1 with assay ID 111143) and POLB with assay ID 147200) (Roche Diagnostic GmbH, Mannheim, Germany). The reference gene was the β-Actin housekeeping gene. The expression analyses were performed using the 2^{ΔΔCt} formula with Light Cycler 480 software program (version 1.5.0 SP4) (Roche Diagnostics Ltd., Rotkreuz, Switzerland). Gene expression levels were measured in the control group, pre-treatment ET group, and post-treatment ET group (who had received 8 weeks of propranolol treatment).

**Statistical analysis**

Statistical analysis was performed with the IBM SPSS Statistics 22 software (IBM SPSS Statistics for Windows, Version22.0. Armonk, NY: IBM Corp. Released 2013). Histograms, Quantile-Quantile Plots, and Shapiro-Wilks tests were used for evaluating the normal distribution of the data. The Wilcoxon rank test was used for pre-post treatment evaluation. Mann Whitney U test was used to evaluate the difference between the control group and the patient group. The Chi-square test was used to compare the distribution according to the gender of the participants. A p<0.05 significance level was accepted.

**Ethical clearance**

Informed consent was obtained from all subjects who agreed to participate in the study. Ethics committee approval was obtained from Erciyes University Ethical Committee of Clinical Studies (2019/82).

---

**Figure 1. Flow diagram of the participants**

---

Assessed for eligibility (n=70)

> Ineligible (n=0)

> Stratified

> Essential tremor (n=35)

> Allocated to propranolol

> Follow-up for 8 weeks

> Analyzed

> Healthy controls (n=35)

> Allocated to control
Seventy people were stratified into two groups, namely the ET patient group (n=35) and the healthy control group (n=35). The ET subjects were given propranolol for 8 weeks (Figure 1). At baseline, there was no significant difference in age and sex (gender) between the ET and control groups (Table 1). A significantly lower \(\text{PARP1}\) gene expression level was found in the pretreatment ET group than in the control group (\(p<0.001\)). \(\text{POLB}\) gene expression was significantly higher in the pre-treatment ET group compared to the control group (\(p<0.05\)) (Table 1). \(\text{POLB}\) gene expression was also significantly higher in the pre-treatment group than in the post-treatment group (\(p<0.001\)) (Table 2). Propranolol significantly reduced \(\text{POLB}\) expression, but the decrease in \(\text{PARP1}\) gene expression was not significant.

**RESULTS**

In our study, the ET patient group showed statistically significant changes in \(\text{PARP1}\) and \(\text{POLB}\) expression levels, in the values compared with the healthy control group. Propranolol significantly reduced \(\text{POLB}\) expression, but the reduction in expression of the \(\text{PARP1}\) gene was not significant. Essential tremor, affecting 4% of individuals aged 40 and over, is one of the most common tremor diseases within the globe. The disease can be either a component of different neurodegenerative diseases or an isolated disease.\(^{18}\) The molecular mechanism of ET is still unclear because there is no specific diagnostic marker for ET. The disease has clinical similarities with some diseases such as dystonia and Parkinson’s disease (PD) and can also be seen together with other neurological diseases such as restless leg syndrome.\(^{19, 20}\) Coexistence with other diseases causes difficulties in the diagnosis of ET.

Twin studies have shown that not only genetic factors, but also environmental factors take part in the etiology of the disease. It was shown in one study that monozygotic twins (60-93%) have greater concordance compared to dizygotic twins (27-29%). According to twin studies, the inheritance of ET has been reported to be between 45% and 90%.\(^{20, 21}\) The genetic structure of ET is quite complex. The genetic etiology of ET comprises both monogenic and multifactorial inheritance. Only a few regions of sensitivity have been identified in the etiology of ET.

**DISCUSSION**

In our study, the ET patient group showed statistically significant changes in \(\text{PARP1}\) and \(\text{POLB}\) expression levels, in the values compared with the healthy control group. Propranolol significantly reduced \(\text{POLB}\) expression, but the reduction in expression of the \(\text{PARP1}\) gene was not significant. Essential tremor, affecting 4% of individuals aged 40 and over, is one of the most common tremor diseases within the globe. The disease can be either a component of different neurodegenerative diseases or an isolated disease.\(^{18}\) The molecular mechanism of ET is still unclear because there is no specific diagnostic marker for ET. The disease has clinical similarities with some diseases such as dystonia and Parkinson’s disease (PD) and can also be seen together with other neurological diseases such as restless leg syndrome.\(^{19, 20}\) Coexistence with other diseases causes difficulties in the diagnosis of ET.

Twin studies have shown that not only genetic factors, but also environmental factors take part in the etiology of the disease. It was shown in one study that monozygotic twins (60-93%) have greater concordance compared to dizygotic twins (27-29%). According to twin studies, the inheritance of ET has been reported to be between 45% and 90%.\(^{20, 21}\) The genetic structure of ET is quite complex. The genetic etiology of ET comprises both monogenic and multifactorial inheritance. Only a few regions of sensitivity have been identified in the etiology of ET.

**Table 1. Distribution of demographic characteristics, \(\text{PARP1}\) and \(\text{POLB}\) at baseline**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Essential tremor group (n=35)</th>
<th>Control group (n=35)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>36.71 ± 11.55</td>
<td>35.11 ± 10.59</td>
<td>&gt;0.05( ^{*})</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>24</td>
<td>16</td>
<td>&gt;0.05( ^{*})</td>
</tr>
<tr>
<td>Female</td>
<td>11</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>(\text{PARP1})</td>
<td>0.32 ± 0.18</td>
<td>0.46 ± 0.16</td>
<td>&lt;0.001( ^{#})</td>
</tr>
<tr>
<td>(\text{POLB})</td>
<td>0.71 ± 0.58</td>
<td>0.46 ± 0.13</td>
<td>&lt;0.05( ^{*})</td>
</tr>
</tbody>
</table>

Note: \(\text{PARP1}\): poly (ADP-ribose) polymerase 1; \(\text{POLB}\): DNA polymerase beta; \( ^{*}\) Mann-Whitney U test; \( ^{\#}\) Chi-square test

**Table 2. \(\text{PARP1}\) and \(\text{POLB}\) expression before and after treatment in the ET group**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Before treatment (n=35)</th>
<th>After treatment (n=35)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\text{PARP1})</td>
<td>0.32 ± 0.18</td>
<td>0.32 ± 0.21</td>
<td>&gt;0.05( ^{5})</td>
</tr>
<tr>
<td>(\text{POLB})</td>
<td>0.71 ± 0.58</td>
<td>0.35 ± 0.20</td>
<td>&lt;0.001( ^{5})</td>
</tr>
<tr>
<td>FTM-TRS Grade</td>
<td>2.4 ± 0.9</td>
<td>1.9 ± 0.7</td>
<td>&lt;0.01( ^{5})</td>
</tr>
</tbody>
</table>

Note: \(\text{PARP1}\): poly (ADP-ribose) polymerase 1; \(\text{POLB}\): DNA polymerase beta; FTM-TRS: Fahn Tolosa Marin tremor rating scale; \( ^{5}\) Wilcoxon rank test
Genome-wide association studies (GWAS) revealed the complex inheritance of ET. The ETM1, ETM2 and ETM3 genes were found to be more effective in familial ET. On the other hand, the SLC1A2, LINGO1, CTNNA3, PPARGC1A and STK32B genes were observed to be effective in sporadic ET. In whole genome sequencing (WES) studies, rare variants in the FUS, HTRA2, TENM4, NOS3, KCNS2, HAPLN4, USP6 genes have been associated with ET with monogenic inheritance.\(^{(22)}\) Liao et al.\(^{(23)}\) demonstrated with PheWAS, a phenome-wide association study, that propranolol treatment can alter the expression level of genes.

Propranolol is one of the recommended first-line treatment options for essential tremors. This drug is a non-selective beta-adrenergic receptor antagonist and is given in divided doses three times a day.\(^{(24)}\) β-adrenoceptors belong to the family of G-protein-coupled receptors, with the β1 and β2-adrenoceptors being expressed in the brain. Central β2-adrenoreceptor blockage is postulated to have favorable effects on tremors.\(^{(25)}\)

Poly (ADP-ribose) polymerase-1 (PARP1) has DNA binding and ADP-ribosylation effects. Through these features, PARP1 constitutes the main part of the cellular repair and defense program mechanism. Poly (ADP-ribose) may also affect some transcription factors, which control the expression of inflammatory mediators, such as nuclear factor kappa B (NFkB). On the other hand, increased PARP1 activation may cause fatal effects on cells by the release of apoptosis-inducing factor (AIF) and NAD\(^+\) and ATP reduction. Even though PARP1 is part of the genome protection mechanisms, hyperactivity of PARP1 can be harmful to the cell. Excessive activation of PARP1 has been linked to several biological processes including aging, neurodegeneration, and parthanatosis. Parthanatos is a caspase-independent programmed cell death pathway because of nuclear translocation of the mitochondria-associated AIF.\(^{(26)}\) Excessive activation of PARP1 has been implicated in several neurodegenerative premature aging disorders such as XPA, CSB, and ATM, and the etiology of stroke.\(^{(27,28)}\)

Poly (ADP-ribose) polymerase-1 has a role in the pathogenesis of Parkinson’s disease (PD). This information can be supported by the presence of much PARP in the nuclei of dopaminergic neurons of the substantia nigra in patients with PD.\(^{(29)}\) Excessive \(\text{PARP}\) activation, which causes DNA damage, occurs with age in wild-type \(\text{C. elegans}.\)\(^{(28)}\) Multiple studies have shown that increased activation is associated with aging, neurodegeneration, and metabolic disorders.\(^{(27)}\) Inhibition of PARP1 provides prominent neuroprotection in animal models; furthermore, it has been determined that PARP1 has a physiological role in the regulation of alpha-synuclein expression. It has also been shown that variants involved in the PARP1 gene promoter decrease the risk of PD and postpone the initial age of the disease.\(^{(30)}\)

The ability of PARP1 to function simultaneously as both catalytic and acceptor proteins has led to conflicting data.\(^{(31)}\) α-synuclein (α-syn) is a presynaptic protein that accumulates and causes loss of neuronal function in PD. Injection of this protein inhibits PARP-1 activity in the cerebral cortex and hippocampus of rat models.\(^{(32-34)}\) The nitric oxide pool that is released after the application of α-syn and NO-mediated caspase-3 activation may play a role in the reduction of \(\text{PARP-1}\) activity.\(^{(35,36)}\)

Both PD and essential tremors are neurodegenerative diseases with tremors. In our results, the expression level of the PARP1 gene in ET was lower than in the control group. The reason for this result can be related to the fact that PARP1 has a significant role in the DNA repair mechanism and the loss of functionality of this gene will be effective in the development of ET. After propranolol treatment, no significant difference was observed in the expression of PARP1. It is particularly important to conduct studies considering PARP targeted therapy to understand the details of the PARP1 catalytic mechanism and regulation.
DNA polymerase β is essential for the base excision DNA repair (BER) pathway, which clears the genome of apurinic/apyrimidinic (AP) regions.\(^{37}\) Apurinic/apyrimidinic sites could be detrimental for cells because they have mutagenic and/or cytotoxic effects. During the repair of AP regions, DNA pol-β participates in synthesis and lyase activities. Due to the crucial role of POLB in sequence conservation, POLB is considered critical for cellular survival. The role of POLB has been confirmed with POLB null mouse cells, that were more sensitive to agents which damage DNA.\(^{38}\)

Publications are proving that endoreduplication occurs during cell division in patients with PD and Alzheimer’s disease (AD). The molecular control mechanisms of endoreduplication in the neurodegeneration process are still confused. Excessive expression of POLB promotes rotenone-induced endoreduplication and this is associated with maintenance of the G2 state. POLB is overexpressed during dopaminergic neuronal death, which is associated with cell cycle re-entry in rotenone-based animal models. Therefore, excessive POLB expression caused by rotenone increases genome instability in dopaminergic cells, through induction of endoreduplication. Increased POLB and re-entry of the cell cycle may be associated with the loss of dopaminergic neurons. To better understand the relationship between POLB and endoreduplication, we need more detailed studies, especially using in vivo models.\(^{39}\)

In our study, the POLB expression level was observed to be higher in ET patients compared to the control group. These results are like those seen in the literature in patients with AD and PD. After propranolol treatment, the POLB expression level was lower than in the pre-treatment group. This clearly shows that propranolol treatment reduces the effects of POLB in ET, considering the recovery status of the patients.

In our study, we examined PARP1 and POLB gene expressions in the blood of ET patients. Considering that ET affects the cerebellar, brain stem, thalamic, and cortical pathways, animal studies can be conducted, and our results can be compared by looking at the expression differences between blood and brain tissue. The number of patients is one of the important limitations of our study. In future studies, looking at the expression levels of these genes with more subjects and in patients treated with different concentrations of propranolol will increase the reliability.

**CONCLUSION**

This study demonstrated that POLB gene expression was significantly higher in the pre-treatment group than in the post-treatment group. Gene expression levels and recovery status of patients can be followed by creating groups treated with different propranolol levels, and these are conceived as commencing strategies for emerging novel treatments.

**ACKNOWLEDGMENT**

Erciyes University supported the study as project TSA-2019-8891.

**CONFLICT OF INTEREST**

The authors declare no competing interests.

**CONTRIBUTORS**

NK and MD developed the theory and concept of the study. SK and HA designed the study. MD, MM, MG, and EK investigated and supervised the findings of this work. NK wrote the manuscript with support from NG, NT, SK, and MD. NG and NK performed the statistical analyses. MD, HA and SK verified the analytical methods. NT, NG, and SK made the literature search. MG, MM and EK performed clinical studies. NK analyzed and interpreted the patient data. All authors discussed the results and contributed to the final manuscript. All authors read and approved the final manuscript.
REFERENCES


