Research Article

Yuan Gao, Li Ding, Jiang Liu, Xiaoyan Wang, Qiang Meng*

Exploring the diagnostic markers of essential tremor: A study based on machine learning algorithms

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Abstract: Essential tremor (ET) is a common neurological disorder with a difficult clinical diagnosis, primarily due to the lack of relevant biomarkers. The current study aims to identify possible biomarkers for ET by screening miRNAs using machine learning algorithms. In this investigation, public datasets and our own datasets were used to examine the ET disorder. The ET datasets originated from public sources. To generate our own dataset, high-throughput sequencing analyses were performed on ET and control samples from the First People’s Hospital of Yunnan Province. Functional enrichment analysis was employed to identify the potential function of differentially expressed genes (DEGs). Using datasets from the Gene Expression Omnibus database, Lasso regression analysis and support vector machine recursive feature elimination were used to screen potential diagnostic genes for ET. To identify the genes responsible for the final diagnosis, area under the curves (AUCs) of the receiver operating characteristic was examined. Finally, an ssGSEA representing an ET immune landscape was created. The sample exhibited expression profiles that corresponded with six genes in the public database. Three diagnostic genes were discovered with AUCs >0.7 that can distinguish ET from normal data: APOE, SENP6, and ZNF148. Single-gene GSEA indicated that these diagnostic genes were closely associated with the cholinergic, GABAergic, and dopaminergic synapse networks. The immune microenvironment of ET was also affected by these diagnostic genes. According to the findings, these three DEGs (APOE, SENP6, and ZNF148) may successfully differentiate between samples from ET patients and normal controls, serving as a helpful diagnostic tool. This effort provided a theoretical foundation for elucidating the pathogenesis of ET and raised hopes of overcoming the diagnostic difficulty of ET clinically.

Keywords: essential tremor, diagnosis markers, high-throughput sequencing, immune microenvironment, functional enrichment analysis

1 Introduction

Essential tremor (ET) is a widely prevalent movement disorder that is characterized by bilateral tremors in the upper limbs during both postural and kinetic activities. It has been suggested that ET is among the most frequently occurring neurodegenerative conditions [1–3]. Clinical symptoms and epidemiology in ET appear to be related. For instance, a study discovered that the incidence of ET has two peaks: early-onset (before the age of 25) and late-onset (after the age of 65) [4]. Positive family history and sensitivity to alcohol are more frequent characteristics of young-onset ET. A higher incidence of dementia has been related to late-onset ET [5]. A meta-analysis conducted globally revealed that the prevalence of ET was 0.4–0.9% across all age groups, while it was 4.6–6.3% in populations aged 65 years and above [6,7]. In addition to tremor, other symptoms of ET include gait and balance impairment, moderate cognitive deficiency, psychiatric symptoms, and hearing loss [8–11]. Recently, the term ET-Plus has been used to characterize these soft signs [12,13].

The pathogenesis of ET is complex and inconclusive due to the involvement of multiple genetic and environmental etiologies. The precise etiology and pathogenesis of ET remains uncertain. Numerous studies in academic literature
have indicated the significant influence of genetic factors [14]. The etiology of ET is supported by the prevalence of positive family history of tremor in patients with ET, indicating the involvement of genetic factors. It is estimated that genetic factors contribute to ET in a range of 20–90% of patients. Additionally, genetic anticipation, which refers to the earlier onset of tremor in the next generation, has been observed in ET patients. Furthermore, twin studies have shown higher concordance rates of ET for monozygotic twins compared to dizygotic twins [15,16]. The diagnosis of ET poses a challenge due to its diverse clinical manifestations, resulting in a high rate of misdiagnosis [17,18].

At present, the clinical diagnosis of ET heavily relies on the subjective clinical evaluation of medical professionals, and a minimum disease duration of 3 years is typically necessary for diagnosis [19]. Globally, there is a severe shortage of experienced neurologists. Therefore, researchers are focusing on incorporating objective measurement indices into the auxiliary diagnosis of ET [20]. Neuroimaging and neurophysiology technologies provide new ideas for confirming the specific pathogenesis of ET. High-precision invasiveness diagnosis, such as 123I-FP-CIT SPECT, has been determined to be the most effective diagnostic instrument in this respect; however, only developed nations can afford it due to its high cost and high consumption [21]. The International Parkinson and Movement Disorder Society (IPMDS) standardized the diagnostic criteria for ET, stating that a 3-year history of isolated action tremor in the absence of any other neurologic condition is required [19]. In order to accurately diagnose a patient, doctors meticulously collect clinical information, including historical features (age at onset, family history), tremor characteristics (body distribution, activation condition), related indicators, and laboratory testing (electrophysiology, imaging, and scales). Due to their age and poor mobility, many ET patients may be unable to see a doctor right away, which would delay receiving an accurate diagnosis and prompt treatment. To help with diagnosis and to distinguish between diagnoses at the early stage of ET, various sensitive and specific detection indexes are still anticipated. As a result, noninvasive auxiliary solutions that are low cost and high efficiency are a new hotspot for ET early diagnosis.

The ET has been linked to cerebellar function, according to the research. The cerebellar degeneration hypothesis is now regarded as a significant player on the ET stage, encompassing movement disorders, cognitive disorders, and affective disorders [22,23]. According to certain scholars, a minimum of 27 miRNAs participate in the regulation of gene expression after transcription and are implicated in the processes of apoptosis and necrosis in cerebellar neurons [24]. Microarrays and bioinformatics analytical tools have been extensively utilized for the identification of differentially expressed miRNAs in ET over the years [25]. A number of miRNAs have been documented to exhibit anomalous expression in individuals with ET, and could potentially have clinical implications [26,27]. In addition, miRNAs have been suggested as potential non-invasive biomarkers for the diagnosis, prognosis, and treatment response of various neurodegenerative conditions, including Parkinson’s disease (PD) and Alzheimer’s disease (AD) [28].

The field of bioinformatics has undergone significant advancements, enabling the screening of a greater number of genes in a more efficient and precise manner compared to traditional experimental research, which is often costly and time-consuming. Bioinformatics analysis has the potential to offer exploratory predictions at a reduced cost, thereby informing subsequent biological experiments and clinical applications [29]. The Hub gene, due to its significant connectivity within the gene expression network, is believed to have a crucial impact on the advancement of the disease [30]. In earlier investigations, hub genes were frequently discovered using the STRING (Search Tool for the Retrieval of Interacting Genes) or cytoHubba program [30]. However, the quality of the screening process and the reproducibility of the experiment are compromised when researchers are allowed to use their personal preferences to determine whether the top 5 or 10 of total differential expression genes (DEGs) should be chosen as hub genes [31]. Various machine learning (ML) approaches have recently been introduced to bioinformatics analysis in an effort to reduce this type of inaccuracy, and it has been demonstrated that doing so improves the accuracy and stability of the screening procedure [32]. As a normalized linear regression method, the least absolute shrinkage and selection operator (LASSO) regression can disregard unimportant features and construct a sparse and easily interpretable model to prevent overfitting. With the support vector machine recursive feature elimination (SVM-RFE) method, the support vector machine is incorporated into the recursive feature elimination approach and its inherent feature selection function is used to continuously screen important features. In several sectors, the combination of LASSO and SVM-RFE algorithms has demonstrated adequate sensitivity and accuracy. High classification performance is achieved by this model, which is recognized to remove irrelevant information effectively [33,34]. Additionally, the most effective techniques currently employed in studies on miRNA biomarkers include bioinformatics and biological tools [35].

In order to assess the potential of the miRNAs to serve as ET biomarkers, this study screened miRNAs based on ML methods utilizing sequencing data from both public databases and the own dataset. Three diagnostic genes, APOE, SENP6, and ZNF148, all with area under the curves
(AUCs) >0.7, were found to have significant diagnostic values for distinguishing ET from normal samples. The synaptic signaling transmission and immunological microenvironment of neurons were thought to be closely related to these diagnostic genes. The present study employed an innovative bioinformatics approach to identify putative biomarkers for ET, which could potentially enhance the accuracy of clinical diagnosis.

2 Materials and methods

2.1 The public data source

The Gene Expression Omnibus (GEO) database, which can be accessed at https://www.ncbi.nlm.nih.gov/geo/ as of November 1, 2022, is a publicly available international repository that contains functional genome datasets generated through high-throughput microarray and next-generation sequencing techniques. The National Center for Biotechnology Information is responsible for the creation and maintenance of this database [36]. This database contains virtually all gene expression assay data that is useful for scientific investigation [37]. Twenty normal samples and 32 ET samples were included in the GSE134878 dataset, which was downloaded for this investigation from the GEO database. For the primary analysis of this study, this dataset was used.

2.2 Patient preparation

The Department of Neurology at the First People’s Hospital of Yunnan Province, China, recruited three patients with ET. All patients underwent clinical and neuropsychological examinations, brain magnetic resonance imaging, and thyroid function evaluations in accordance with IPMDS [19] criteria. Exclusion criteria included concurrent or recent exposure to tremorgenic drugs, hyper- and hypothyroidism, hyperparathyroidism, physiologic and psychogenic tremor, premorbid clinically significant psychiatric disorders, alcohol and narcotic addictions, inflammatory diseases, cancer, chronic diseases, and a history of major surgery. All of the patients had a 3-year history of bilateral upper limb tremors. In addition, three age- and gender-matched healthy subjects were recruited as controls [38]. Fahn Tolosa Marin scale was utilized to evaluate the severity of tremor [39]. The Chinese version of the Montreal Cognitive Assessment (score 0–30) was used to evaluate cognitive function. The Beck Depression Inventory (score 0–63) was used to assess affective symptoms.

Informed consent: Informed consent has been obtained from all individuals included in this study.

Ethical approval: The research related to human use has been complied with all the relevant national regulations, institutional policies and in accordance with the tenets of the Helsinki Declaration, and has been approved by Medical Ethics Committee of the First People’s Hospital of Yunnan Province (YYLH005, February 2019).

2.3 RNA-seq analysis

Peripheral blood mononuclear cells (PBMCs) were isolated from ET patients and healthy controls by Ficoll density gradient centrifugation and Lymphoprep (Stemcell, USA). Following their separation, PBMCs were lysed using a TRIzol Reagent (Invitrogen, USA) and kept at ~80°C for further processing. Then, according to the manufacturer’s recommendations, total RNA was isolated using a mirVana miRNA Isolation Kit (Ambion, Foster City, CA). The quantity of RNA was assessed using a NanoDrop 2000 (Thermo Fisher Scientific, Waltham, MA), while RNA quality was evaluated using an Agilent Z100 bioanalyzer (Agilent Technologies, Santa Clara, CA). Purified libraries were created using Illumina TruSeq Stranded Total RNA Sample Preparation Kits (Illumina, San Diego, CA) in accordance with the manufacturer’s instructions, and their quantities were determined using an Agilent 2100 bioanalyzer and a Qubit 2.0 Fluorometer (Life Technologies, Carlsbad, CA). Libraries were utilized to create a cluster using the cBot software. Subsequently, the cluster underwent sequencing utilizing the Illumina HiSeq 2500 platform, which is located in San Diego, CA. The sequencing procedures were conducted by Origin-Biotech Inc. (Ao-Ji Bio-Tech, Shanghai, China).

2.4 Analysis of DEGs

The differential expression files, which had undergone DESeq2 processing, were acquired directly from the GEO database (Table S1). DEGs were identified based on the statistical criteria of a significance level of \( P < 0.05 \) and a false discovery rate threshold of \( <0.25 \). The \( t \)-test assay was utilized to obtain the expression pattern of the DEGs in the own dataset, as indicated in Table S2.

2.5 Functional enrichment analysis

The DEGs underwent functional enrichment analysis using the clusterProfiler package, specifically through the utilization of Gene Ontology (GO) and Kyoto Encyclopedia of
Genes and Genomes (KEGG). Cellular component (CC), biological process (BP), and molecular function (MF) were the three basic categories used in the GO analysis. The KEGG signaling pathway was considered a pre-established gene set, and subsequently, gene set enrichment analysis (GSEA) was conducted on individual diagnostic markers to identify the specific pathway associated with each diagnostic marker. The adjusted (adj.) \( P < 0.05 \) values were regarded as significant for all statistical analyses.

### 2.6 LASSO and SVM-RFE algorithms

DEGs with consistent expression trends in their own and GSE134878 datasets were regarded to be potential ET-related DEGs. The LASSO and SVM-RFE algorithms were utilized to filter candidate diagnostic genes for ET from among the candidate DEGs associated with ET. The glmnet (version 3.0) and e1071 (version 1.7-3) R package (version 3.6.0) were utilized to conduct LASSO and SVM-RFE, respectively. The genes that were identified by both algorithms were considered as potential diagnostic genes. By comparing the AUC of the receiver operating characteristic (ROC) curves, the efficacy of candidate diagnostic genes in differentiating ET samples from normal samples was evaluated. A gene with AUCs greater than 0.7 in both the GSE134878 and own datasets was considered the diagnostic gene.

### 2.7 Single sample GSEA (ssGSEA)

ssGSEA is a GSEA deconvolution algorithm that translates gene expression profiles into quantitative fractions of immune cells in a single sample. The GSVA function in the R package was utilized to evaluate the distribution of 24 immune cell subtypes in each sample of the GSE134878 dataset [42]. Since no sample included evidence of the expression of the IL3RA-only gene in pDC cells, these cells were specifically excluded of all subsequent analyses.

### 2.8 Statistical analysis

Pearson correlation analysis was employed to calculate correlations, and a correlation was deemed significant if it satisfied the criteria of \(|\text{correlation (cor)}| \geq 0.3\) and \(P < 0.05\). The regulatory networks in which diagnostic genes may be engaged were revealed using Ingenuity Pathway Analysis (IPA). The statistical analyses were primarily conducted using the R package (version 3.6.0). Statistical analyses were conducted with a significance level of \(P < 0.05\), indicating statistical significance.

### 3 Results

#### 3.1 Filtering of ET-related DEGs and unveiling of their potential functions

A total of 231 DEGs related to ET were identified in the GSE134878 dataset, as shown in Table S3. Figure 1a shows that of these, 171 were up-regulated and 60 were down-regulated. Furthermore, the expression pattern of the DEGs in the GSE134878 dataset was demonstrated through a heat map, as depicted in Figure 1b.

The potential roles of DEGs in the pathogenic progression of ET were then investigated using GO analysis (Figure 1c; Table S4). The DEGs identified in the BP category were observed to have a close association with synaptic transmission and neuronal conduction. Specifically, the DEGs were found to be involved in various processes such as modulation of chemical synaptic transmission, regulation of trans-synaptic signaling, regulation of synaptic plasticity, positive regulation of synaptic transmission, neuron projection guidance, positive regulation of neuron projection development, neurotransmitter secretion, and positive regulation of neuron differentiation. The identified genes were observed to have significant involvement in cellular structures such as the postsynaptic density, asymmetric synapse, postsynaptic specialization, and neuron-to-neuron synapse. In addition, it was observed that four terms exhibited significant enrichment in the MF category. These terms include tau protein binding, tubulin binding, tau-protein kinase activity, and microtubule motor activity. In addition, the KEGG analysis revealed that the DEGs were primarily associated with various biological processes such as vascular smooth muscle contraction, Salmonella infection, axon guidance, and oxytocin signaling pathway (as shown in Figure 1d and Table S5).

#### 3.2 Identification of candidate ET-related DEGs

The expression pattern of the aforementioned DEGs was verified between a collection of three normal and three ET
samples using t-tests. A total of 231 DEGs were identified, out of which eight DEGs exhibited differential expression in the dataset under investigation. Subsequently, six out of the eight DEGs exhibited consistent expression patterns in the GSE134878 dataset. The study found that the genes \textit{EFR3A}, \textit{FAM169A}, \textit{SENP6}, and \textit{ZNF148} exhibited overexpression in ET, while \textit{APOE} and \textit{NISCH} showed repression in ET, as depicted in Figure 2. These genes were identified as potential ET-related DEGs and were selected for further analysis (Figure S1).

Figure 1: (a) Volcano plot showing DEGs for the ET and control groups (black dots denote no significant difference, red dots denote differential expression that was up-regulated, blue dots denote differential expression that was down-regulated). (b) Heat map of DEGs between the ET and control groups. (c) Results of GO enrichment analysis for DEGs. (d) Results of the investigation of KEGG enrichment for DEGs.
Figure 2: (Continued)
3.3 Screening for ET diagnostic markers and assessment of their diagnostic validity

Two distinct methodologies were utilized to identify the most effective diagnostic markers for ET by analyzing potential DEGs associated with ET. The LASSO algorithm was utilized to identify a set of characteristic genes, which consisted of five DEGs, as depicted in Figure 3a and b. On the other hand, the SVM-RFE algorithm was employed to select a set of four DEGs, as illustrated in Figure 3c and d. Through the process of overlapping the biomarkers selected by the two algorithms, a total of four DEGs were identified,
as illustrated in Figure 3e. These DEGs were subsequently identified as potential feature genes for the classification of ET. In this study, a cohort of 52 test samples sourced from the GSE134878 dataset, alongside six validation samples from an own dataset, were classified into ET and normal groups based on the utilization of four potential feature genes. The results of the ROC curve analysis indicate that APOE, SENP6, and ZNF148 achieved a high level of classification accuracy in both the GSE134878 dataset (with all AUCs greater than 0.7, as shown in Figure 3f) and the researcher's own dataset (with all AUCs equal to 1, as shown in Figure 3g). Therefore, these genes have been validated as diagnostic markers for ET. Figure 1a depicts the annotation of ET diagnostic markers' expression patterns in the GSE134878 dataset.
3.4 Single-gene GSEA for ET diagnostic markers

The KEGG signaling pathway was utilized as a pre-gene set, and GSEA was used to reveal the signaling pathways implicated in each of the three diagnostic markers. The study found that APOE was associated with 15I enriched pathways. The three most enriched pathways were identified as “Ribosome,” “Herpes simplex virus 1 infection,” and “Coronavirus disease – COVID-19,” as shown in Figure 4a and Table S6. The study identified 32 pathways associated with SENP6, with the “Ubiquitin mediated proteolysis,” “Complement and coagulation cascades,” and “AD” pathways being the most significant, as depicted in Figure 4b and Table S7. The study identified 145 pathways associated with ZNF148 through GSEA. Among these pathways, “Endocytosis,” “Thyroid hormone signaling pathway,” and “Sphingolipid signaling pathway” were found to have the strongest correlation with ZNF148, as depicted in Figure 4c and Table S8. Remarkably, the investigation revealed a significant correlation between the genes and specific synaptic pathways, namely “GABAergic synapse” [43] (APOE, ZNF148), “Cholinergic synapse” [44] (ZNF148), “Dopaminergic synapse” [45] (ZNF148), and “Serotonergic synapse” [46] (ZNF148). Moreover, these diagnostic markers were implicated in diverse neurodegenerative disease pathways, including Huntington’s disease (HD), PD, amyotrophic lateral sclerosis, AD, and spinocerebellar ataxia. The findings indicate that the aforementioned genes could potentially play a role in the development of ET and other associated neurodegenerative disorders. Additionally, Figure S1 illustrates a potential regulatory network implicated in the diagnostic markers identified by IPA.

3.5 Immune landscape analysis of ET patients

The study conducted a functional enrichment analysis of DEGs and diagnostic markers associated with ET. The results indicated a strong association between these genes and the immune response, including pathways such as the cAMP signaling pathway, cell adhesion molecules, antigen processing and presentation, Th17 cell differentiation, ECM–receptor interaction, T cell receptor signaling pathway, and AMPK signaling pathway. Inspired by this, the ssGSEA algorithm was used to estimate the proportion of immune infiltrating cells between normal and ET patients in the GSE134878 dataset. The findings indicate that the fractions of Tgd and Th1 cells were the only statistically significant differences observed between the two groups. Specifically, Tgd cells were found to be less infiltrated in ET patients, while Th1 cells were more widely distributed in ET patients (Figure 5a and Figure S1). Subsequently, the correlation between diagnostic markers and immune infiltrating cells was calculated using Pearson correlation analysis (Figure 5b and Table S9). The findings indicate a significant positive association between APOE and Tgd (cor = 0.56, P = 1.32 × 10^-5), macrophages (cor = 0.42, P = 0.00185), TFFH (cor = 0.37, P = 0.006253), and eosinophils (cor = 0.36, P = 0.009681). The findings suggest that SENP6 exhibits a negative correlation with cytotoxic cells (cor = -0.36, P = 0.008547) and THF (cor = -0.33, P = 0.015557), whereas a positive correlation was observed with Th helper cells (cor = 0.38, P = 0.005584). Only CD8 T cells exhibited a positive correlation with ZNF148 (cor = 0.39, P = 0.004593) (Figure S3).

The study conducted a significant association between ZNF148 and the expression of PD-L1 and the PD-1 checkpoint pathway in cancer. As a result, an investigation was carried out to determine the expression levels of 29 immune checkpoint molecules in both normal and ET patients, as illustrated in Figures S2 and S3. The results revealed that CD47 and TNFSF4 exhibited high expression levels in patients with ET, while LGALS9, PDCD1, and TNFRSF14 were highly expressed in the normal group, as depicted in Figure 5c. The results of Pearson correlation analysis indicated that CD47 exhibited a strong positive correlation with SENP6 (cor = 0.35, P = 0.010676). Additionally, LGALS9 demonstrated significant positive and negative correlations with APOE (cor = 0.36, P = 0.008381) and SENP6 (cor = -0.37, P = 0.006177), respectively. However, no significant correlation was observed among TNFSF4, TNFRSF14, and the diagnostic markers, as shown in Figure Sd and Table S10 (Figure S2).

4 Discussion

This study represents a novel investigation into the diagnostic markers of ET utilizing ML algorithms, with the aim of elucidating the underlying pathogenesis. During the preliminary investigation, it was observed that three diagnostic genes played a significant role in the diagnosis process and were implicated in complex pathophysiological mechanisms. The aforementioned discoveries have facilitated a redefinition of our comprehension of the mechanism and have provided clarification regarding the complexity of clinical manifestations.

The manifestation of tremors, as observed in clinical settings, can be attributed to the interaction between the cerebellar thalamus, cortex, and muscle, resulting in involuntary muscle contractions [47,48]. Several neuroimaging
Figure 4: (a) Single-gene GSEA for APOE, (b) single-gene GSEA for SENP6, and (c) single-gene GSEA for ZNF148.
studies have demonstrated the existence of functional, metabolic, and structural defects in the cerebellum of individuals with ET [49–51]. The postmortem literature has been expanding and has identified microscopic abnormalities in the brain of individuals with ET. These abnormalities are primarily focused on the Purkinje cells and synaptic transmission [52–54]. According to certain researchers, compared to controls, ET patients had significantly lower dendritic complexity and spine density [55]. The Purkinje cells, acting as efferent neurons within the cerebellar cortex, receive primary excitatory inputs from the climbing fibers of olivary cells via synapses. Subsequently, they transmit information through synapses to establish a one-to-one relationship [56,57]. The cerebellum of individuals with ET has been found to exhibit anomalies in Glu synapses and GABA synapses, particularly in terms of synapse density, as indicated by postmortem studies [58–60]. The aberrant formation of Purkinje cells and their synapses, leading to cellular remodeling and degeneration, has been found to give rise to novel or atypical cortical circuits in individuals with ET, thereby contributing to the manifestation of associated clinical symptoms [61,62].

The potential functions of DEGs in the pathogenic course of ET were investigated via GO and KEGG pathway enrichment analysis. Despite the lack of clear correlation between DEGs and the nervous system in the KEGG analysis, it is postulated that the DEGs primarily impact synaptic transmission and neuronal conduction, as indicated by the results of the GO analysis (Figure 1c and d). The findings of the analysis indicated a close correlation between the DEGs and the functions of tau protein and microtubule-associated protein. These DEGs were found to be responsible for inducing abnormalities in the cytoskeleton. These genes were also discovered to play crucial roles in CCs such postsynaptic density, asymmetric synapses, and postsynaptic specialization. This finding explained the axonal and dendritic structural alterations in compromised Purkinje cells, as well as the underlying degenerative process involving the Purkinje cells and/or their microenvironment [63].

Three diagnostic genes were identified as the optimal markers for ET diagnosis from candidate DEGs associated with ET. The three diagnostic genes were found to have significant involvement in the process of cellular structure remodeling and metabolic function anomalies specifically in Purkinje cells. The GSEA analysis revealed that three diagnostic genes were significantly linked to diverse neurodegenerative disease pathways across distinct synapses, as depicted in Figure 4.
The APOE gene, which controls multifactorial/complex processes resulting in the early death of neurons, had been thoroughly investigated in ET. The APOE gene is known to regulate the metabolism of amyloid precursor protein and the accumulation of amyloid-beta (Aβ). It has also been found to promote the hyperphosphorylation of tau-protein, reduce choline acetyltransferase activity, increase oxidative processes, modify inflammation-related neuroimmunotrophic activity, and alter synapse structure, among other effects [64,65]. An elevation in insoluble Aβ42 levels was previously observed in the cerebral parietal cortex of all individuals with ET, as well as in the cerebellar white matter of the majority of ET cases [66]. This accumulation of cerebellar Aβ42 indicated continuous neurodegeneration in a subset of ET patients and clarified the cognitive impairment of ET-plus patients [65]. The findings of this investigation indicate a decrease in the expression of APOE in individuals with ET, as demonstrated in Figure 2. The results were in agreement with prior research indicating that the reduction of plasma APOE levels could serve as a peripheral biomarker. This decrease may indicate an increase in ET brains, which has also been noted in individuals with AD [67].

The close association between SENP6 and hereditary stability has been suggested as a role that is evolutionarily conserved in regulating chromatin dynamics and genome stability networks. This is achieved through the maintenance of a balance in the chromatin residency of protein complexes [68]. The results of the GSEA conducted on SENP6 indicated that the pathways of “Ubiquitin mediated proteolysis” and “AD” were notably prominent, as illustrated in Figure 4b. Extensive research has been carried out suggesting that SENP6 was responsible for modifying synaptic transmission and neuronal excitability in neurons via small ubiquitin-related modifier, which is compatible with the finding of this work [69]. ZNF148 has been linked to various cellular processes, such as cell proliferation, differentiation, and programmed cell death, and has been identified as a potential tumor suppressor [70,71]. In research for HD, as well as a progressive neurodegenerative disorder, the researchers described that ZNF148 directly interacted with three known-HD genes (BCL2, CASP6, and IRS2) [72]. Additionally, ZNF148 had been identified as a previously unknown yet significant transcription factor in the corpus callosum’s development [73,74]. The evidence suggests that ZNF148 may have a significant impact on abnormal neuronal proliferation or migration, as well as abnormal axonal growth or guidance [74]. The aforementioned results indicate that SENP6 and ZNF148 could potentially contribute to the development of ET and other associated neurodegenerative disorders.

It had been established that an imbalance in GABAergic inhibition can result in excitotoxicity and the upregulation of glutamatergic and cholinergic neurons, ultimately leading to the manifestation of delirium and involuntary tremors in patients with ET [75,76]. These three genes were found to be significantly linked to synapses and were observed to participate in the gene network of neurotransmitters. Learning disabilities and memory deficits were observed as a consequence of abnormal APOE gene expression in GABAergic interneurons [77,78]. The post-translational modifications occurring at both presynaptic buttons and post-synaptic terminals were found to regulate the activity of SENP6 [69]. SENP6 controlled a number of synaptic functions, including the degradation of target proteins, which is crucial for the creation and recall of memories [79]. Furthermore, it was demonstrated that ZNF148 exhibited the ability to stimulate the promoter of SLC6A1, a transporter responsible for the termination of GABA activity through the elimination of GABA from the synaptic cleft [80]. Conversely, certain antiepileptic drugs (AEDs) have potential for employment in the clinical treatment of ET, including Mysoline (Primidone), Klonopin (Clonazepam), Ativan (Lorazepam), and Valium (Diazepam). The pharmacological targets of these AEDs were mainly focused on glutamatergic and GABAergic synapses [43]. The involvement of APOE and ZNF148 in GABAergic synapses has been observed, indicating that these synapses may serve as potential targets for the treatment of ET using AEDs.

It was observed that the DEGs associated with ETs were linked to the immune response, as depicted in Figure 5a–d. Despite ET being classified as a neurodegenerative disorder, increasing evidence has suggested that the activation of lymphocytes and microglia may contribute to neuroinflammation in ET [81–83]. The findings indicate that there were significant differences in the fractions of Tgd and Th1 cells between normal individuals and those with ET. Specifically, Tgd cells were found to be less infiltrated in ET patients, while Th1 cells were more widely distributed in ET patients, as depicted in Figure 5a. Th1 cells stimulated macrophages by means of interleukin 2 (IL-2), which caused the release of interleukin 8 (IL-8) [84]. A recent study has reported that patients with ET exhibit considerably elevated serum levels of IL-8 in comparison to control patients. Additionally, the study found a positive correlation between the severity of tremor and the serum IL-8 level, which further supports our conclusion [81]. Tgd cells have been observed to participate in a multifaceted immune response within the gut–brain axis, which has been shown to exert a substantial impact on the pathogenesis of central nervous system neurodegenerative disorders, including PD and AD, through immune
cell-mediated mechanisms [85,86]. The intestinal microflora in the context of autoimmune disease may also have an impact on the pathological and clinical outcome of ET [87].

The findings of our study indicate a significant positive association between APOE and Tgd, macrophages, TFF, and eosinophils. The study revealed that SENP6 exhibited an inverse association with cytotoxic cells and TFF, whereas it displayed a direct association with T helper cells. The results presented in Figure 5b indicate a positive correlation between ZNF148 and CD8 T cells. The relevant research was examined, revealing that APOE has an impact on physiology and pathophysiology at various levels. These levels include the inhibition of CD4+ and CD8+ lymphocytes, regulation of macrophage function, modulation of inflammation and oxidation, and reduction of IL-2 production [88,89]. The inhibition of neuroinflammation was achieved through the dampening of nuclear factor kappa-B (NF-κB) activation by SENP6 [90]. ZNF148 was found to exert an influence on immunoreactivity in T cells through its regulatory role in the expression of immune molecules [91]. The findings of this study suggest an indirect association between neuroimmune activity and the onset of ET.

The study conducted an in-depth analysis of the expression levels of immune checkpoint molecules in patients with ET and explored the correlation between immune checkpoint molecules and diagnostic markers, representing truly an innovative approach in the field. In particular, it was discovered that CD47 had a high level of expression in ET patients, and that this expression was both positive and substantially linked with SENP6. The aforementioned results have exposed the fundamental inflammatory mechanisms involved in ET and have also identified SENP6 as a potentially efficacious gene therapy tool that could be further explored in the context of treating ET patients.

5 Conclusions

In conclusion, the findings revealed that these three DEGs (APOE, SENP6, and ZNF148) may effectively differentiate between samples from ET patients and normal controls, hence providing a useful diagnostic tool. The study employed bioinformatic methodology to anticipate the potential functional roles of DEGs and investigate their plausible involvement in the pathogenesis of ET. The finding had the potential to contribute to an improved understanding of the pathogenic processes that ultimately result in ET. However, the validation trial for detecting plasma miRNA expression levels in a large sample size of ET patients and controls has not been conducted due to financial limitations. Subsequently, additional verification experiments will be conducted in the forthcoming work. In addition, consistency of the findings of ML approaches needs to be evaluated on a larger dataset in the future to further establish the universality of ML. The precise biological roles and molecular processes by which these three DEGs contribute to the pathogenesis of ET will also be the subject of future studies.

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