Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/jptcp.v31i8.7472

GDNF AND NEURONAL RESILIENCE IN AUTISM: THE ROLE OF GLIAL CELL-DERIVED NEUROTROPHIC FACTOR IN MODULATING SYNAPTIC PLASTICITY

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ABSTRACT

A complicated neurological disease known as autism spectrum disorder is typified by issues with social interaction, communication, and repetitive behavior. Recent researches indicate that the neural protective mechanisms in ASD may be influenced by neurotropic genes like Glial Cell Line-Derived Neurotropic Factor (*GDNF*). We aim to examine the relationship between neuronal protection and cognitive functioning by crosslinking *GDNF* gene expression, Serum levels with relation to MMSE scores in ASD patients. After study ERC approval (ERC/199/06/02/2021) EDTA blood samples (5 ml each) were drawn from study population (n=140) including 100 ASD patients with more than 30 months of disease course from various clinical settings and 40 healthy controls. The Analytical procedures included nucleic acid extraction, primer design and optimization, and *GDNF*-targeted RT-qPCR expression analysis. To measure cognitive and behavioral deficits, enzyme-linked immunosorbent assays (ELISA) based serum *GDNF* levels (pg/ml) and Minimal Mental State Examination (MMSE) Scores were compared concluding Neuronal protection potential of *GNDF*. In patients with ASD showing, lower serum levels of *GDNF* (8.371±2.38pg/ml) were linked to more severe behavioral and cognitive deficits confirmed by MMSE scores (13.6±3.5) of ASD patients in comparison with control group (27.1± 2.1). Healthy individuals showed higher relative gene fold (11.71) as compared to the ASD patients (5.51). There is a notable decrease in *GDNF* gene expression in people with ASD, which raises the possibility that *GDNF* is important for both cognitive performance and neuronal protection in these people. *GDNF* may be a useful biomarker for identifying ASD and comprehending its molecular causes and treatment approaches.

Keywords: Neurobiology, *GDNF*, Neuroprotection, *Autism*, Cognitive impairment, Biomarkers penalization
Short Title: *GDNF's* Therapeutic Potential Vs Autism Patients

INTRODUCTION

ASD is a multifaceted neurodevelopmental disorder marked by repetitive behaviors, speech challenges, and issues with social interaction. [1] The pathophysiology of ASD is largely dependent on neurotrophic proteins and genetic variables, as recent research has shown. For this reason, studying genes such as *GDNF* is very important. It facilitates long-term potentiation (LTP), a key mechanism underlying memory consolidation. [2] Synaptic plasticity refers to the ability of synapses to strengthen or weaken over time in response to increases or decreases in their activity. This dynamic process is fundamental to learning, memory, and the adaptive changes in the brain's neural networks. The neurotrophic factor (NTF) protein family, of which *GDNF* is a part, is well-known for having a substantial influence on the survival, differentiation, and maintenance of neurons. This component is essential to neurodevelopmental processes because it promotes neuronal growth and survival throughout childhood and adolescence. [3]

The presence of *GDNF* in the synaptic microenvironment enhances neuronal resilience, enabling adaptive changes in response to environmental stimuli. *GDNF*'s neuroprotective qualities are particularly crucial in halting the neuronal degeneration of neurons seen in a number of neurological disorders, including ASD thus playing a vital role in synaptic plasticity control. [4] Additionally, *GDNF* has anti-inflammatory properties, which are extremely pertinent in light of the mounting data that connects chronic inflammation to the etiology of ASD. GDNFs are pivotal in promoting the survival and differentiation of dopaminergic neurons, directly influencing synaptic strength by involving complex signaling pathways, including the activation of the RET receptor tyrosine kinase. *GDNF* may help reduce some of the neurological symptoms connected to ASD by reducing inflammation. [5]

Reduced *GDNF* levels have been linked to higher levels of inflammation and oxidative stress, both of which are often made worse in people with ASD. These elements may exacerbate existing neural damage and impair cognition. [6] Numerous potential genes linked to ASD have been found by genome-association studies (GWAS). Of these, *GDNF* has emerged as a new gene of interest because of its regulatory and neuroprotective properties. [7] Dysregulation of GDNF signaling has been linked to synaptic dysfunction in neurodegenerative diseases, highlighting its therapeutic potential. GDNF influences the structural plasticity of neurons by modulating dendritic spine morphology and synapse formation. The neurophysiological functions of *GDNF* include enhancing neurogenesis, synaptic plasticity, and general brain function—all of which are essential for preserving behavioral and cognitive health in people with ASD. [8]

The roles of the neurotrophin family—which includes *GDNF*, *GDNF*, NT-4/5, and NT-3—in maintaining and promoting neuronal survival have been well investigated. Particularly, *GDNF* interacts with receptors that are important for several neurophysiological processes necessary for brain health and function, including TrkA, TrkB, TrkC, and the low-affinity receptor p75NTR. [9] The MMSE (Thirty points) is a brief questionnaire that is widely used to assess cognitive impairment in clinical research. It assesses cognitive abilities like math, memory, and orientation and yields a numerical score that may be used to monitor improvements over time. [10]

Our study attempts to establish a relationship between the degree of cognitive and behavioral deficits as determined by MMSE scores and the expression levels of *GDNF* opening up new possibilities for early detection and focused treatment approaches. Through comprehending the molecular processes behind ASD and recognizing pivotal elements like *GDNF*, the aid in the advancement of more efficacious therapies and assistance plans for those diagnosed with ASD can be delivered.

MATERIALS AND METHODS

After Study ERC approval from Institutional Board and conformity to the outlined principles in the Declaration of Helsinki, A total of about 140 EDTA blood samples (5ml each) of ASD patients (n=100) and healthy controls (n=40) were collected after written informed consent from clinically diagnosed cases Autism in various clinical settings and Neurology OPDs in Tertiary hospitals from Islamabad and Lahore, Pakistan.

Inclusion criteria was age (>40y), male and females, healthy controls, Type II DM, confirmed cases of Neurodegenerative disorder, Autism. Exclusion criteria was no clinical history, lack of diagnostic tests record, refused informed consent and secondary Diabetics. These samples under standard Transport Protocols were transported to the MOU signed diagnostics Labs for storage until further processing. *GDNF* serum levels in the samples was measured by ELISA using a kit for the quantitative determination of serum *GDNF* levels (IHUADPNKTC # IH0556) by manufacturer's protocol. QIAgen blood kit (QIAamp#56604) was used to isolate genomic DNA from peripheral blood according to the manufacturer's protocol.

DNA quality check is essential for reliable molecular analysis. Techniques such as UV spectrophotometry, fluorometry, and gel electrophoresis were used to assess the concentration, purity and integrity. Optimal absorbance 260/280 and 260/230 ratios, visualizing intact bands, and confirming amplifiability through PCR ensure high-quality DNA. For running gel of desired DNA samples, 1.5% gel was prepared as per standard procedure. The gel apparatus conditions were set on 70 Volts for about 40 minutes. Finally, the results were analysed on a SS Doc.

Amplification was performed on Thermocycler (Bio-Rad-114) using PCR Master Mix (Thermofisher 4426518) in 40 µL of RNAse-free water containing 0.35 µM primers. The PCR conditions used were 4 min of initial denaturation at 95°C, 1 minute of denaturation at 94 °C, 15 seconds of annealing at 53°C, and 1 minute of extension at 72°C. The PCR cycle was repeated 40 times with a final extension at 72 °C for 10 min, followed by cooling to 4°C.

The primers were designed on a serial cloner by using the consensus CDS sequence of specific genes from the NCBI database and then primer specificity or universality was checked by primer-BLAST or BLASTn respectively. Primers were optimized using a gradient PCR thermocycler (Bio-Rad T100-Thermocycler, USA) to get the best optimal temperature. Their melting temperatures (Tm) and amplicon properties were optimized. Sequences of the designed primers (forward and reversed) are shown in Table 1**.**

Table 1. Sequences of Designed Primers

***GDNF* Primers Pair**	***GDNF* sequence (5\` to 3\`)**
Froward	TGCTGGCCTAATAGAGTGCA
Reverse	TGCACTCTATTAGGCCAGCA

Demographic data was plotted through bar charts and frequencies of relative morbid conditions. Using SPSS One way ANOVA was done to find variance amongst the samples. Statistical significance of $p>0.05$ was considered.

RESULTS

140 blood samples (61 male and 79 female) were taken for the investigation including 40 healthy controls. All verified instances of autism were evaluated using the Minimal Mental Score Examination (MMSE) and *GDNF* levels (pg/ml) as shown in Table 2.

Table 2: demographics, *GDNF* levels and MMSE scores of Study Population (n=140)

Gender	**Average age ±Standard deviation**	**Cases (%)**
Male	52.4±8.5	61
Female	56.1±4.6	79

Variables	**ASD patients** (n=100)	**Healthy Controls** (n=40)	**T-test** (p-value)
GDNF levels (pg/ml)	8.371±2.388	15.371± 3.64	0.013
MMSE scores	13.6±3.5	27.1± 2.1	0.008

The MMSE questionnaire was utilized to assess the mental state of the patients. The individuals were classified as having less/no cognitive impairment (24 or higher score) and severe cognitive impairment (0–15) based on their MMSE scores. The medical experts who created the exam scored the results based on the predetermined criteria that were included in the questionnaire. Figure 1 below illustrates how the severity-based scoring interpretation is determined;

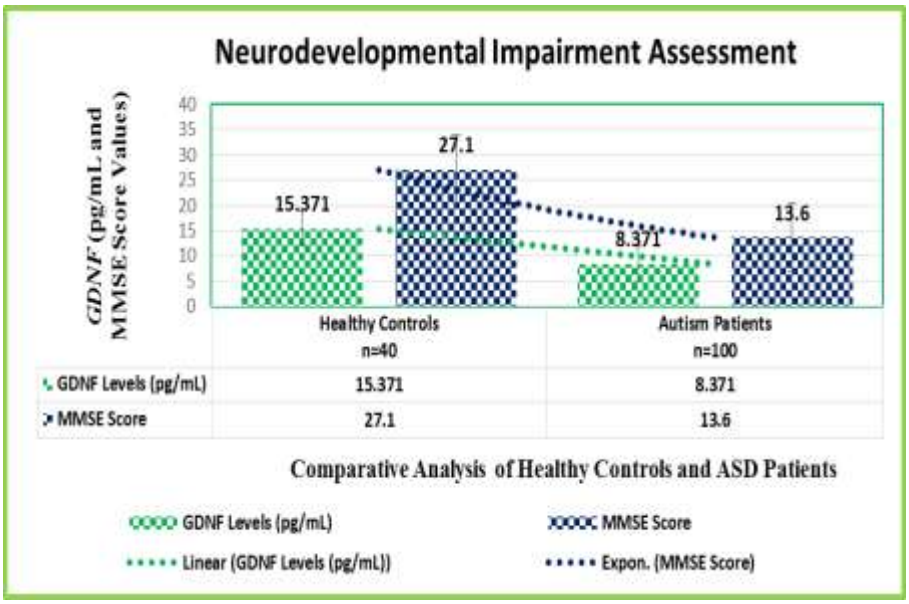

Figure 1: *GDNF* based assessment for ASD and MMSE analysis

In comparison to healthy individuals, ASD patients showed decreased *GDNF* levels (8.371) and low MMSE (13.6) scoring confirming the phenomenon of cognitive impairment in Autism.

Relative Fold Change of *GDNF* gene in ASD Patients with Reference to Healthy Controls via RT-qPCR:

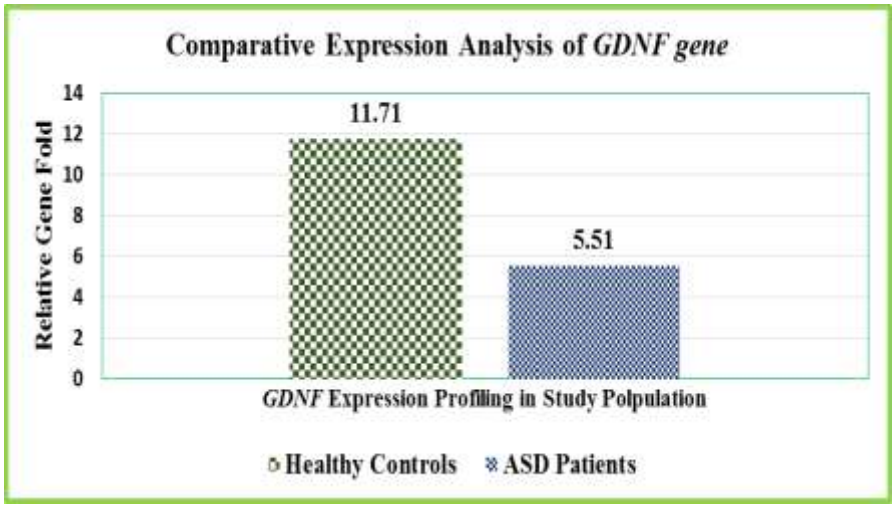

Figure 2: Relative gene fold change in healthy controls and ASD cases.

Our data showed that Healthy individuals possess higher *GDNF* relative gene fold (11.71) as compared to the ASD patients (5.51).

DISCUSSION

Investigating the expression of Glial Cell Line-Derived Neurotrophic Factor (*GDNF*) genes provides important insights into putative neural defense mechanisms in the fight to comprehend and treat Autism Spectrum Disorder (ASD). ASD causes problems in cognition, behavior, and social interaction by affecting several brain areas and neural networks. [11] An individual's risk of acquiring cognitive impairments is increased by conditions such as oxidative stress and neuroinflammation. Our study's main focus, the gene *GDNF*, has been linked to these conditions, changing the mental functioning abilities of people with ASD. According to our research, *GDNF* gene malfunction increases oxidative stress and neuroinflammation, which in turn hastens the cognitive decline of ASD patients. [12] Significant differences in *GDNF* expression levels were seen in the recorded data of individuals with ASD, indicating a possible biomarker function for *GDNF* in the diagnosis and understanding of ASD pathogenesis. The behavioural and cognitive abnormalities linked to ASD are mostly caused by reduced neuroplasticity and synaptic dysfunction, which are caused by decreased *GDNF* expression.

Studies have indicated that an overabundance of *GDNF* may cause amyloid precursor proteins to accumulate in the golgi apparatus, obstructing their appropriate sorting to late endosomal compartments where amyloid-beta is produced. [13] On the other hand, when it is underexpressed, *GDNF* levels drop, which exacerbates synaptic dysfunction and neuronal atrophy. Our results support these observations since we found that individuals with ASD had lower levels of the *GDNF* gene expression, which had similar pathogenic effects. [14] We found that individuals with ASD had reduced expression of *GDNF* in our study. In several instances, the Mini-Mental State Examination (MMSE) score—which is frequently used to assess cognitive impairment—was less than 10, indicating significant cognitive abnormalities. In our ASD patients, the relative fold change determined by real-time PCR revealed a fold drop of around 4.1, suggesting that the *GDNF* gene was not being expressed as much. Additionally, diabetics with ASD showed a fold drop of 4.9, indicating a correlation between worse cognitive performance and reduced *GDNF* expression in these individuals. [15]

These findings support those of previous research showing reduced *GDNF* activity in individuals with neurodegenerative conditions such as Alzheimer's disease. High *GDNF* gene activity has been linked to amyloidogenesis, the production of amyloid protein, which in turn causes amyloid plaques to develop in the brain and worsen neurodegenerative illnesses. [16] *GDNF* plays a critical role in pathogenic traits including neuroinflammation and synaptic dysfunction, which are shared by ASD and AD despite being separate illnesses. [17] A vital factor in ASD, a complex disorder, is the decrease in *GDNF*. There is a wealth of data demonstrating the correlation between lower levels of *GDNF* and improved cognitive performance. Reduced *GDNF* expression is elevated in the brains of AS patients, which is linked to neuronal atrophy, compromised neuroplasticity, and synaptic dysfunction. [18] Neurotrophic support is further compromised by the downregulation of pro-*GDNF*, the precursor of *GDNF*, which occurs concurrently with the drop in *GDNF*. [19]

Additionally, peripheral indicators including decreased *GDNF* expression in peripheral blood mononuclear cells (PBMCs) and low serum levels of *GDNF* have been identified as possible diagnostic markers. The relationship between decreased serum levels of *GDNF* and cognitive deterioration highlights the significance of *GDNF* as a peripheral biomarker for ASD. Comprehending the complex interplay of *GDNF*, ASD, and peripheral indicators provides insights into the underlying pathophysiology and opens up new options for therapeutic treatments and diagnostic improvements. [20,21] A potential tactic to slow down cognitive aging and improve the chances of early identification and treatment for illnesses linked to ASD is to target dysregulation of *GDNF*. [22,23] Future research can create targeted therapeutics aiming at restoring *GDNF*'s normal expression and function by concentrating on its neuroprotective capabilities. This might potentially alleviate the behavioral and cognitive symptoms linked to ASD. This method advances our

knowledge of neurodevelopmental and neurodegenerative disorders while also having the potential to enhance the quality of life for those with ASD.

CONCLUSION

According to the findings of this study, the *GDNF* gene has a great deal of promise as a biomarker for identifying and treating autism spectrum disorder (ASD). Early identification of changed *GDNF* expression may enable prompt and focused therapy interventions, therefore slowing the development of behavioral and cognitive deficits in ASD patients. Furthermore, the distribution of *GDNF* mRNA in peripheral blood mononuclear cells emphasizes a systemic element, emphasizing the usefulness of peripheral markers in reflecting changes in the central nervous system. Enhancing *GDNF* dysregulation may help individuals with ASD live better lives and lessen the socioeconomic burden that comes with the disorder.

FUTURE DIRECTIONS

Elucidation of Mechanistic Pathways: Future research should focus on unraveling the precise molecular mechanisms through which GDNF modulates synaptic plasticity in the context of autism. Understanding these pathways at a deeper level will be crucial for identifying therapeutic targets and optimizing interventions.

Targeted Therapeutic Strategies: Developing specific therapeutic strategies that leverage GDNF to enhance neuronal resilience in individuals with autism is a promising avenue. This includes designing novel drugs or gene therapies that can precisely modulate GDNF signaling to restore or enhance synaptic plasticity.

Longitudinal and Multimodal Studies: Conducting long-term studies that combine genetic, neuroimaging, and behavioral assessments will help establish a more comprehensive understanding of how GDNF influences neuronal resilience over time.

Integration with Personalized Medicine Approaches: Future work should explore the integration of GDNF-based therapies with personalized medicine. By tailoring interventions to the specific genetic and neurobiological profiles of individuals with autism, it may be possible to achieve more effective and individualized treatments.

Exploration of Combinatorial Therapies: Investigating the potential of combining GDNF modulation with other therapeutic approaches, such as behavioral interventions or other neurotrophic factors, could offer synergistic benefits. This approach could enhance overall therapeutic outcomes by addressing multiple facets of synaptic plasticity and neuronal resilience.

Ethical and Safety Considerations: As GDNF-based therapies progress towards clinical application, it is essential to address ethical and safety concerns, particularly regarding long-term effects and potential off-target impacts. Establishing clear ethical guidelines and safety protocols will be crucial for responsible development and use.

AUTHORSHIP CONTRIBUTION STATEMENT

This inter-collaborative Comprehensive Study involved significant inputs from all Authors of various Institutes as per ICMJE criteria. Moreover, the Authors named as Saima Hameed (Department of Biosciences, COMSATS University Islamabad, Pakistan), Amna Naheed Khan (Department of Biosciences and Bioinformatics, Capital University of Science and Technology, CUST Islamabad) and Sabahat Fatima (Department of Biochemistry, Gujranwala Medical College, GMC Gujranwala Pakistan) contributed equally to work.

CONFLICT OF INTERESTS

The authors declare no conflict of interest.

GRANT SUPPORT & FINANCIAL DISCLOSURES

The authors declare No Grant disclosure.

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