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Synapsin Gene Mutations in Idiopathic Generalized Epilepsy Patients in Pakistan

Mehwish Riaz[1], Nadeem Sheikh*[1], Tayyaba Saleem[2], Muddasir Hassan Abbasi *[3,4]

[1]Cell & Molecular Biology Lab, Institute of Zoology, University of the Punjab, Q-A- Campus, Lahore, 54590, Pakistan.
[2]Department of Neurology, University Medical Center, Georg-August-Universitäts, Robert-Koch-straße 40, 37075, Göttingen, Germany
[3]Guangzhou Women and Children's Medical Center, Guangzhou Medical University-China
[4]Department of Zoology, University of Okara, Okara, Pakistan.

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* nadeem.zool@pu.edu.pk *muddygcs@gmail.com

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Abstract

Background: *Epilepsy is a heterogeneous, multifactorial disease in which genetics plays an important role. The synapsin (SYN2) gene has been considered one of the important epilepsy-associated candidate genes that plays an important role in epileptogenesis. Although various studies conducted in different countries have evaluated the association between the onset of epilepsy and the presence of the SYN2 rs3773364 variant, such an association has not yet been evaluated in Pakistan. Therefore, the aim of this study was to determine the genetic association of the SYN2 rs3773364 variant with the onset of epilepsy in the population of Pakistan.*

Materials and Methods: *Blood samples of 150 epilepsy patients and 150 healthy controls who were recruited in our previous research on the genetic association of GABA receptor gene polymorphisms with epilepsy were employed for the isolation of genomic DNA for further analysis through PCR and DNA sequencing method.*

Results: *Both homozygous and heterozygous mutations were detected in epileptic patients. Single-site analysis showed significant differences in the genotypic and allelic frequencies of SNP rs3773364 in both epileptic patients and controls, with a p-value of 0.0003.*

Conclusion: *The SNP rs3773364 of SYN2 significantly contributes to the epilepsy onset in Pakistan. The outcomes suggested that the SYN2 gene might play a critical role in the progression of epilepsy among patients in Pakistan.*

Key words: *Epilepsy, Genetic association, Idiopathic generalized epilepsy, SYN2*

Introduction

Epilepsy is one of the most common neurological disorders, which is characterized by the sudden interruption of normal neuronal signaling (Fukata & Fukata, 2017). It is estimated that there are 70 million people worldwide who suffer from epilepsy, with 85% of these cases occurring in developing countries (Muhigwa et al., 2020; Qian et al., 2022; Vannini et al., 2020). A recent study in Pakistan revealed that there is an overall epilepsy prevalence of 10/1000 people, a total of 2 million epileptic patients, and it accounts for $1/10^{th}$ of the global epilepsy burden (Nadeem et al., 2018; Tanveer et al., 2022). The pathogenesis and etiology of epilepsy are still poorly understood, and genetic factors are believed to play an important role in the disease. After the discovery of the first human gene associated with epilepsy, almost 977 genes, including synapsin family genes, have been associated with different types of epilepsy (Wang et al., 2017).

Overall, 140 proteins are found to be engaged in synaptogenesis, their transport, and recycling, in which synapsin is the most abundant family of (~1%) phosphoproteins that regulate multiple steps in these processes (Chesnokova et al., 2025; Turner et al., 2021). Synapsins encoded by *SYN1-3* genes are neuronal phosphoproteins in the CNS and PNS. These genes and proteins are crucial for the formation of synaptic vesicles and transmission of neurotransmitters between presynaptic and postsynaptic membranes (Bonanomi et al., 2005; Forte et al., 2024; Messa et al., 2010). Normally, synapsin binds the synaptic vesicle to the actin cytoskeleton, which limits the release of the neurotransmitters. However, when the neurons are activated, synapsin is phosphorylated

by Ca^{2+}/calmodulin–dependent protein kinase II (CaMKII), which in turn mobilizes the vesicles towards the presynaptic membrane, leading to the fusion of the vesicle and the release of the neurotransmitters (Chiappalone et al., 2009; Hilfiker et al., 2005; Mirza & Zahid, 2018; Schwark et al., 2022). Epilepsy occurs when the concentration of synapsin is perturbed due to a mutation in SYN genes, and vesicles continue releasing neurotransmitters, leading to hyperexcitation and seizures (Cesca et al., 2010; Etholm & Heggelund, 2009; Mirza & Zahid, 2018).

Genes (*SYN1-3*) encoding synapsin family phosphoproteins (Syn I-III) are situated on sex and autosomal chromosomes (X, 3p25, and 22q12-13, respectively) in higher vertebrates. Synapsin I and II are found mainly in the hippocampus and cortical regions of the brain. Though Synapsin III has been cloned from human DNA, it has unique expression and functional characteristics that differentiate it from the other members of the synapsin family (Kielland et al., 2006; Lakhan et al., 2010; Porton et al., 2011). These proteins together comprise for 9% of vesicle proteins, and their splicing gives rise to two isoforms (a and b) for Syn I and II that are similar at A, C, and E domains but not at the B domain (Molinaro et al., 2015; Porton et al., 2011; Song & Augustine, 2015).

Gene *SYN2* encoding synapsin II protein, bears 17 exons and spans almost 140kb region at the position 3q25.2 (Cavalleri et al., 2007; Farisello et al., 2013). Syn II protein was reported to be involved in the regulation of neurotransmitters, synaptic vesicle formation, and their fusion on postsynaptic neurons (Forte et al., 2024; Kielland et al., 2006; Mirza & Zahid, 2018). This gene was reported to follow an X-linked inheritance pattern affecting more males than females (Sharma et al., 2025).

Genetic studies to find susceptible loci in epilepsy reported that mutations in *SYN2*, one of the major five genes, are associated with epilepsy predisposition (Cavalleri et al., 2007; Saviouk et al., 2007; Sharma et al., 2025). In consideration of the potential association of the *SYN2* (rs3773364) variant with epilepsy, the purpose of the current study was to investigate its association with the development of epilepsy in the Pakistani population.

Materials and Methods

SDS Subjects and Sample processing

Genomic DNA was extracted from blood samples of 150 epileptic and 150 case control subjects that were previously recruited for the identification of the genetic association between GABA receptor gene polymorphisms and epilepsy prediction in Pakistani population study (Riaz et al., 2021). DNA quantity, purity, and quality were analyzed using spectrophotometry and automated electrophoresis.

Molecular and genetic analysis

SNP rs3773364 was chosen for mutational analysis in this cohort since, to our knowledge, no preliminary studies have been reported for the Pakistani population. Previously reported forward and reverse primers for *SYN2* rs3773364 were used (Lakhan et al., 2010), with optimization of the annealing temperature to 58 °C to amplify the PCR product, which was subsequently sequenced commercially.

Genotyped DNA sequence analysis was done using BioEdit sequence alignment editor (7.2.6.1), and any possible mutations were determined. Alignment of DNA sequences was done using NCBI BLAST to compare reference and allelic sequence variations. Hardy-Weinberg equilibrium was used to check deviations from expected frequencies of alleles. Fisher's exact test was used to find out if there is any relationship between the selected single-nucleotide polymorphism (SNP) and epilepsy. Odds ratio and confidence interval were used to find out if there is any relationship between genotype and epilepsy. A p-value of <0.05 was considered significant.

Results and Discussion

Epilepsy is a neurological disorder resulting from overstimulation of the neurons, leading to a significant psychiatric and socioeconomic impact. The present study has been done to ascertain the importance of SYN2 (rs3773364) gene and its association with epilepsy in the Pakistani population, as recent studies have highlighted the importance of this gene and its association with epilepsy. As the rate of consanguineous marriages is high in our population, the likelihood of identifying a correlation with previously reported gene(s) is considerably higher in this population.

All the extracted DNA samples in the current study met the quality standards for DNA quantity ($\geqslant$ 20 ng/µL) and purity (A260/280 ratio 1.7-1.9) as shown by the amplified DNA (146bp) (Figure 1a). A single mutational site corresponding to *SYN2* rs3773364 was identified, with both homozygous and heterozygous variants were observed in epileptic patients, whereas only the wild-type genotype was detected in healthy controls (Figure 1B-D).

The first nonsense mutation (c.1067G>A) in the synapsin gene family (*SYN1*) was identified in a four-generation family with X-linked epilepsy (Garcia et al., 2004). Similarly, a large multicenter study involving 1,528 epileptic patients from UK, Ireland, Finland, and Australia identified a significant association of the A>G polymorphism in the SYN2 gene (rs3773364) with epilepsy in the Irish, Finnish, and UK populations but not in the Australian cohort (Cavalleri et al., 2007). These findings support a notion that intronic A>G polymorphism in the *SYN2* (rs37733634) may be a significant factor in the Pakistani population's susceptibility to epilepsy.

An association between epilepsy and SYN2 (rs3773364), located in intron 5 in a North Indian population has shown a higher distribution of the AG genotype of rs3773364 in patients compared with controls, whereas no significant difference was observed in the GG genotype (Lakhan et al., 2010). Similar to this, another study carried out in Iraq also revealed a significant difference in the distribution of the AG genotype, while there were no significant differences for the AA and GG genotypes (Hameedi & Saud, 2021).

Synapsin contribution to the pathophysiology of epilepsy emerged when the synapsin gene was removed from the mice, and that resulted in increased seizure susceptibility and a decreased number of synaptic vesicles (Cesca et al., 2010; Etholm & Heggelund, 2009; Feng et al., 2002). The SYN2 (rs37733634) A>G polymorphism has been linked to idiopathic generalized epilepsy, according to a different study conducted by Prasad and colleagues on the south Indian population (Prasad et al., 2014).

It was observed that epileptic patients had a higher frequency

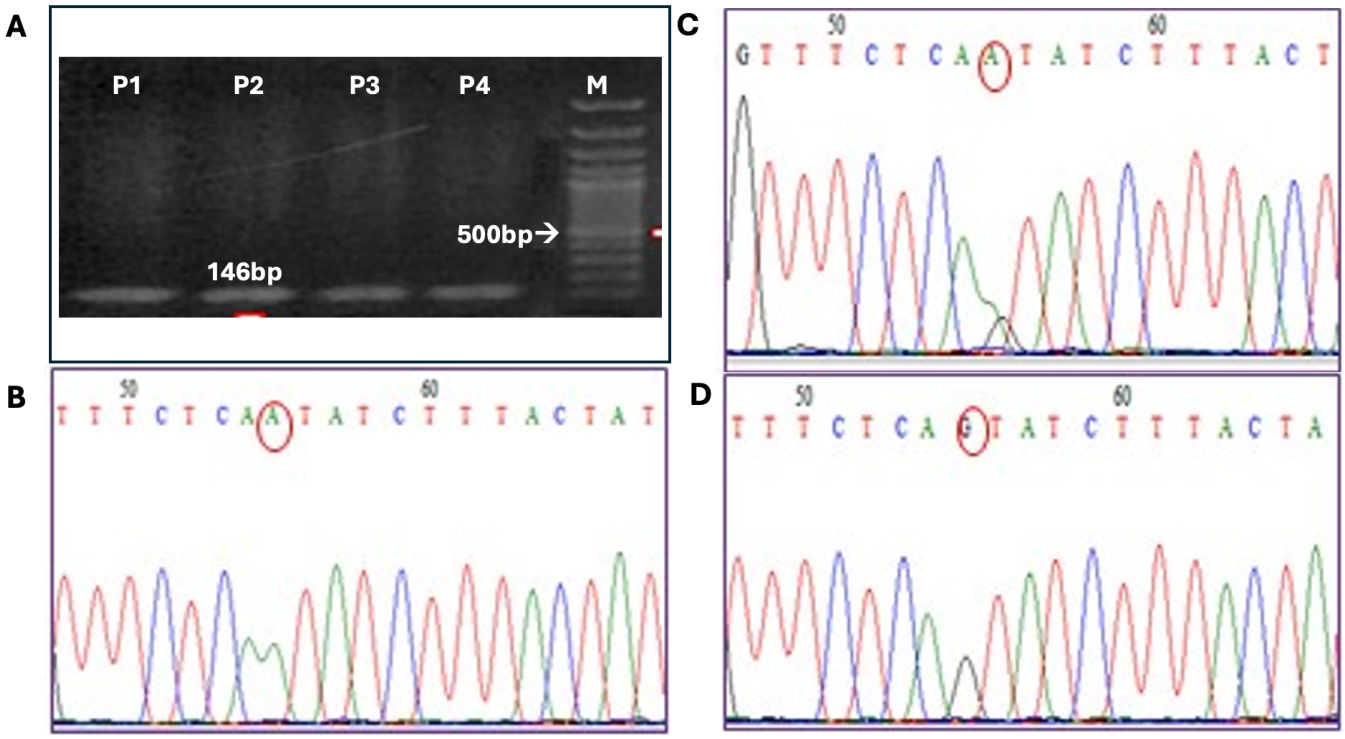

Figure 1: Screening of SYN2gene from the stored blood samples of epileptic subjects. A) PCR amplified band (146bp), electropherogram showing genotype of SYN2 gene: (B) Control genotype AA, (C) Patient genotype AG, (D) Patient genotype GG.

Table 1: Single Site Analysis of SYN2 Allelic variants in subjects

Variant			Frequency		Odds ratio	Fischer's Exact Test
			Patients	Control	95% Confidence Interval	P-value
rs3773364	Allele	A (Reference)	0.61	0.75	0.528736 (0.372705~0.750087)	0.0003**
		G (Altered)	0.39	0.25		
	Genotype	AA	0.42	0.65	-	0.0003**
		AG	0.39	0.21		
		GG	0.19	0.15		

**Level of significance (p<0.01)

(Freq) of variant allele G (0.39) compared to controls (0.25). With a p-value of 0.0003, the Odds ratio (OR) with 95% confidence level indicates that the likelihood of passing it to the following generation was 0.52 times higher. The genotype distribution was associated with epilepsy and conformed to the Hardy-Weinberg Equilibrium (P>0.05). Allelic and genotypic frequencies determined by using BLAST and Hardy–Weinberg equilibrium (HWE) are shown in Table 1.

Although the *SYN2* (rs37733634) A>G intronic mutation does not alter the amino acid sequence, it may change the alternative splicing, leading to changes in the mature protein's shape. Epilepsy has been linked with a number of different mutations within the same gene family, and it has been suggested that this is due to a gain- or loss-of-function, leading to an abnormality of signaling (Fassio et al., 2011).

Conclusion

In conclusion, our case-control study has demonstrated a statistically significant association between the SYN2 (rs3773364) A>G polymorphism and an increased susceptibility to the onset of epilepsy within the Pakistani population. Therefore, this variant can be considered a risk factor for epilepsy.

Author contributions

Conceptualization: NS and MHA; Methodology: TS; Sample collection: TS; Investigation: TS and MR; Data Curation: TS & MR; Writing—Original Draft Preparation: MR, TA and MHA; Writing—Review & Editing: All authors; Supervision: NS and MHA.

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