

Anti-Gamma Aminobutyric Acid B Autoimmune Encephalitis in an Indian Child with Early-Onset Seizures, Neurodegeneration, and Brain Calcification due to *NRROS* Variation: The First Reported Case Worldwide

Aritra Kapat¹ Alak Pandit² Suman Das² Dilip Kumar Paul¹ Asok Kumar Mandal¹
Ashok Kumar Bala¹

¹Department of Pediatric Medicine, Dr. B C Roy Post Graduate Institute of Pediatric Sciences, Kolkata, West Bengal, India

²Department of Neuromedicine, Bangur Institute of Neurosciences, Kolkata, West Bengal, India

Address for correspondence Suman Das, MBBS, MD, DM, 44 Talpukur Road, Deulpara, Naihati, West Bengal, PIN-743165, India (e-mail: dr.sumands@gmail.com).

J Pediatr Epilepsy 2023;12:109–114.

Abstract

A 1.5-year-old boy presented to us with a history of normal growth and developmental parameters until 6 months of age. However, at 7 months of age, he developed multiple types of seizures consisting initially of complex febrile seizures, followed by afebrile seizures. Multifocal clonic, generalized tonic-clonic, and myoclonic (multifocal and generalized) were the evolving seizure types. He had truncal hypotonia, but his appendicular hypotonia progressed to hypertonia over the next few months and further to decorticate posturing. Brain magnetic resonance imaging (MRI) showed generalized atrophy, predominantly frontotemporal, without any focal signal abnormalities or contrast enhancement. Computed tomography (CT) showed speckled calcification in subcortical white matter. Electroencephalogram showed bilateral frontotemporal epileptiform discharges with secondary generalization. His cerebrospinal fluid had normal cytology and biochemical results but was positive for anti-gamma aminobutyric acid B antibodies. Whole exome sequencing showed likely pathogenic, novel autosomal recessive homozygous variation of *NRROS* gene on chromosome 3 [c.1487G > A (p.Trp496Ter)], which impairs the functioning of anti-inflammatory cytokine transforming growth factor beta, resulting in a proinflammatory state within the central nervous system and thereby promoting autoimmune encephalitis. Parental Sanger sequencing validated the variation in both his parents. He was treated with both pulse methylprednisolone (30 mg/kg/day for 5 days) and intravenous immunoglobulin (2 g/kg), followed by slowly tapering of oral prednisolone and monthly intravenous immunoglobulin infusion (1 g/kg). There was significant reduction in seizure frequency and disappearance of epileptiform discharges from the electroencephalogram. However, the motor and cognitive improvement did not occur, and he had microcephaly and growth failure at the last follow-up. This is the 11th case report of neurodegeneration associated with *NRROS* gene variations, but the first report of autoimmune encephalitis being triggered by the variation in a child.

Keywords

- ▶ microglia
- ▶ *NRROS*
- ▶ SENEBC
- ▶ autoimmune encephalitis
- ▶ TGF-β

received
August 4, 2022
accepted
September 20, 2022
article published online
November 9, 2022

© 2022, Thieme. All rights reserved.
Georg Thieme Verlag KG,
Rüdigerstraße 14,
70469 Stuttgart, Germany

DOI <https://doi.org/10.1055/s-0042-1758147>.
ISSN 2146-457X.

Introduction

Microglia, the long-living innate immune cells in the brain, are hosts to innumerable genes, which encode for a plethora of central nervous system (CNS) disorders—neurodevelopmental, neurodegenerative, neuroinflammatory, and neoplastic.¹ *CSF1R*, *DAP12*, *TYROBP/TREM2*, *USP18*, *IRF8*, and *NRROS* genes have been linked to diverse phenotypes of microgliopathies.² *NRROS* (negative regulator of reactive oxygen species) is a transmembrane protein of the endoplasmic reticulum containing leucine-rich repeats, whose function was first described by Noubade et al in 2014.³ Phagocytes produce reactive oxygen species (ROS) to eliminate invading pathogens. However, tight regulation is needed during inflammatory processes to prevent collateral tissue damage. *NRROS* controls NADPH oxidase 2 (NOX2) degradation and toll-like receptor signaling and activates the latent anti-inflammatory cytokine transforming growth factor beta (TGF- β) by anchoring it over the surfaces of macrophages and microglia.⁴ Thus, *NRROS* regulates ROS generation, thereby minimizing collateral tissue damage. In mice experiments, *NRROS*-deficient phagocytes exhibited elevated ROS production during inflammation, with resultant enhanced microbicidal activity. However, due to oxidative CNS tissue damage, these mice developed fatal autoimmune encephalomyelitis.^{4,5}

Loss-of-function variants in the *NRROS* gene result in infantile-onset encephalopathy characterized by early-onset seizures with neurodegeneration and brain calcification (SENEBAC), publications on which are exceedingly rare. Two articles^{2,4} describing nine cases in 2020 were followed by a recent case report in April 2022.⁶ Ours is the 11th case reported worldwide and the first from India. Moreover, it is the first report to describe associated autoimmune encephalitis in a child with a *NRROS* variation.

Case Report

The index case is of a second-born boy from a second-degree consanguineous marriage. The boy had normal growth and developmental milestones until 6 months. At 7 months, he had his first episode of febrile status epilepticus, followed by recurrent episodes of complex febrile seizures. At 9 months, he had an afebrile generalized tonic-clonic seizure, soon followed by the appearance of myoclonic jerks, which were both multifocal and generalized, and occurred in clusters of five to seven jerks in each cluster. He also had clonic jerks involving both upper limbs. His multiple seizure types progressed in frequency and severity, associated with global developmental regression. His family history was noncontributory to any type of epilepsy.

He presented to our center at 1.5 years of age. There were no dysmorphisms, neurocutaneous markers, abnormal body or urinary odors, or organomegaly. His hospital discharge reports after birth showed normal birth weight and head circumference. He initially had both axial and appendicular hypotonia, followed by the appearance of appendicular hypertonia over the next 3 months, brisk deep tendon

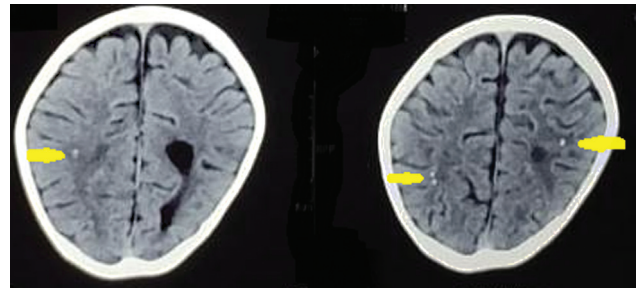


Fig. 1 Noncontrast CT brain showing speckled calcifications in subcortical white matter (marked with yellow arrows).

reflexes, and bilateral extensor plantar responses. Subsequently, he developed decorticate posturing (clinical photographs in ► **Supplemental File 1**, available in online version only). He was treated with multiple antiepileptic drugs (AEDs) such as levetiracetam, clobazam, and valproate, but without any significant benefit.

The complete hemogram, liver, renal, and thyroid function tests, cerebrospinal fluid (CSF) cell count, glucose and protein levels, and plasma lactate and ammonia levels were normal. The CSF autoimmune panel by indirect immunofluorescence test was positive for anti-gamma aminobutyric acid (anti-GABA) B antibody. Noncontrast computed tomography (CT) showed diffuse brain atrophy with punctuated scattered calcifications, predominantly in the subcortical white matter (► **Fig. 1**). Magnetic resonance imaging (MRI) of the brain showed diffuse cerebral cortical and subcortical atrophy with relative sparing of the cerebellum and brainstem without any focal signal abnormalities or contrast enhancement (► **Fig. 2**). The frontotemporal atrophy was more prominent than the parieto-occipital atrophy (► **Fig. 3**). The blood spot tandem mass spectrometry and urinary gas chromatography mass spectrometry were unremarkable for any metabolic disease. Electroencephalogram (EEG) showed predominantly bilateral frontocentrotemporal high-amplitude spike and sharp wave discharges (► **Fig. 4**), with secondary generalization (► **Fig. 5**), indicating encephalopathy. No sleep elements were seen during EEG. With a normal chest skiagram and abdominal ultrasound, other internal organ malformations were excluded. After obtaining parental consent, the next-generation sequencing of the whole exome was performed (► **Supplemental File 1**) to look for genetic etiology.

Viewing the rapidly progressive encephalopathy in the background of anti-GABA B antibody positivity, we treated the child with pulse intravenous methylprednisolone (30 mg/kg/day for 5 days) and intravenous immunoglobulin (IVIg; 2 g/kg). Thereafter, his seizure frequency significantly reduced over the next 1 month. He was discharged on tapering doses of oral prednisolone, sodium valproate (60 mg/kg/day), levetiracetam (60 mg/kg/day), and clonazepam and was planned for monthly IVIg (1 g/kg) regime. The exome sequencing revealed a novel likely pathogenic autosomal recessive homozygous biallelic variation [c.1487G > A (p.Trp496Ter)] located on exon 3 of the *NRROS* gene. Sanger sequencing validated the presence of the variations in heterozygous state

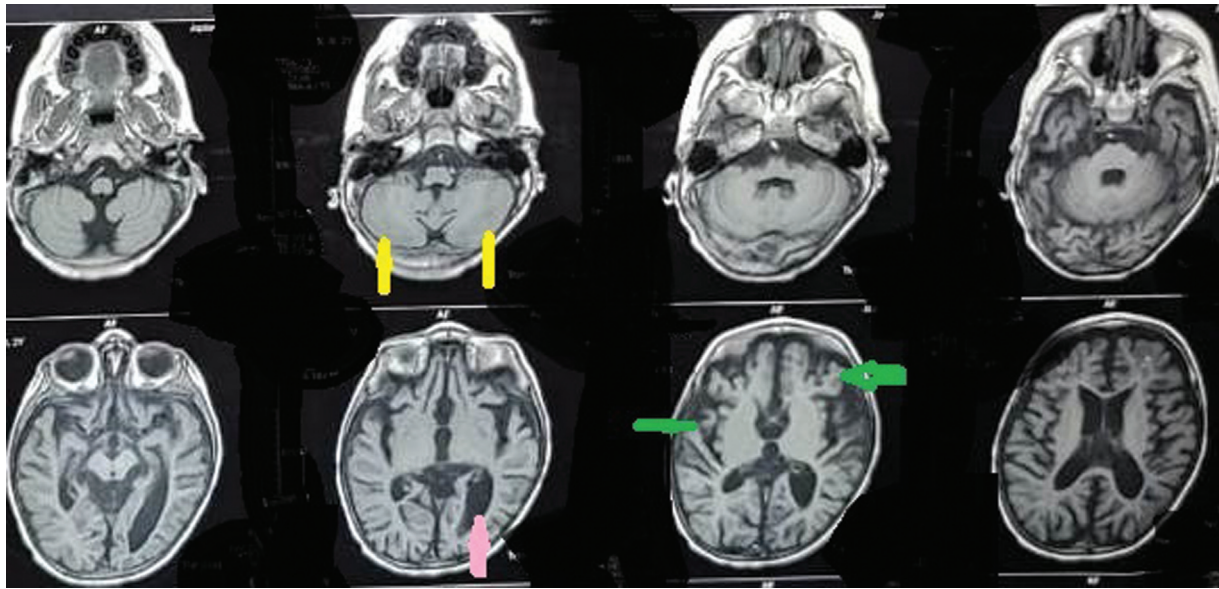


Fig. 2 Noncontrast MRI brain T1 sequence axial section showing diffuse cortical (marked with green arrows) and subcortical atrophy, without any focal signal abnormalities. There is relative cerebellar sparing (marked with yellow arrows). The subcortical atrophy is asymmetrical with greater dilatation of the left occipital horns compared with the right side (marked with pink arrows).

in both the parents (► **Supplemental File 1**, available in online version only).

He has been followed up over the last 8 months. He is currently on his prior AED regimen and oral prednisolone taper (currently 0.5 mg/kg/day) and has received 7 monthly IVIG pulses. Repeat CSF autoimmune profile, done 3 and 6 months after discharge, was negative for anti-GABA B antibody, but MRI brain showed mild progression of atrophic changes. He was seizure-free but significantly disabled with clinical decortication symptoms, without any speech output

or visual tracking. His EEG showed significant improvement, without any epileptiform discharges, but still lacked the normal sleep elements (► **Fig. 6**). At the last visit, his occipitofrontal circumference was 44 cm and his weight was 8.2 kg (both z-scores < -3 of the World Health Organization growth charts).

Discussion

Smith et al described three children with SENEBC (two girls and one boy) from two unrelated families, born out of non-consanguineous marriage, in whom, after initial normal early development, the patients developed refractory seizures and neuroregression between 14 and 18 months, ultimately leading to death by the third year of life.² Histologically, there was widespread neuronal loss in the grey matter with reactive gliosis, neuropil calcification, and foamy macrophage infiltration in the perivascular spaces of the cerebral white matter from the frontal to the occipital cortices and cerebellar white matter.² Dong et al reported six children (three boys and 3 girls) from four unrelated families with seizure onset varying between 9 and 17 months.⁴ Four children had mild-moderate developmental delay from the first year of life, while two had normal initial development with rapid regression after the onset of seizures. Multiple evolving seizure types were described: refractory focal seizures, infantile spasm syndrome, clonic, myoclonic, and complex partial seizures. Only one patient had onset of seizures along a febrile illness, similar to our child. Three children expired at 2 years 2 months, 3 years 3 months, and 4 years 2 months, whereas three children were alive at 20 months, 4 years 6 months, and 9 years 3 months when the article was published. Macintosh et al described the latest case who had ultrastructural defect in the mitochondria when examined by electron microscopy.⁶ In their brain MRIs, these patients

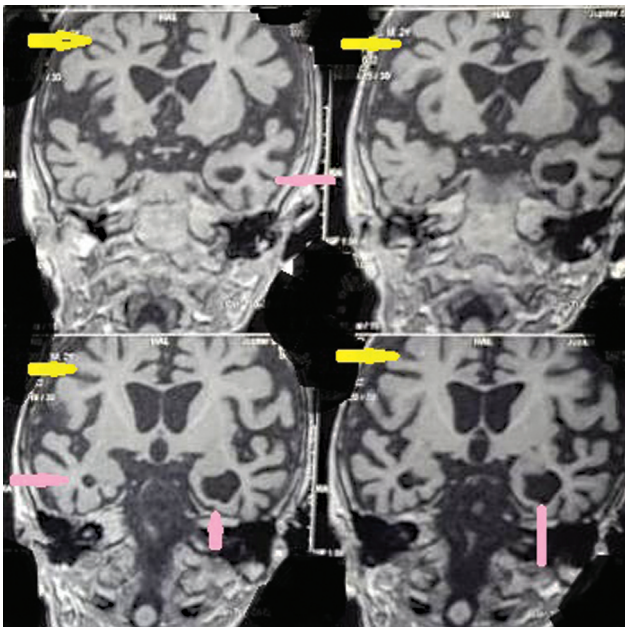


Fig. 3 Noncontrast MRI brain T1 sequence coronal section showing predominant bilateral frontal (marked by yellow arrow) and temporal atrophy with asymmetrical dilatation left > right temporal horns of the lateral ventricles (marked by pink arrow).

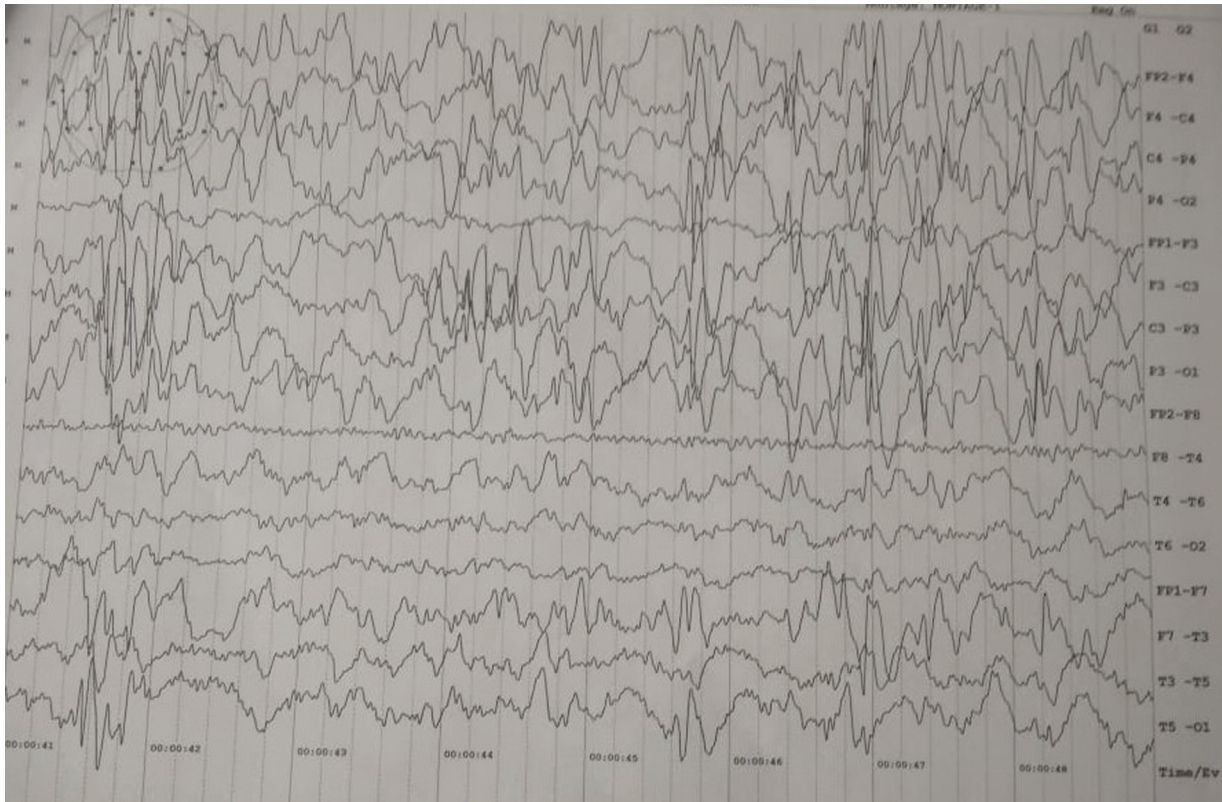


Fig. 4 Bipolar longitudinal montage of sleep EEG at sensitivity 7.5 Hz/mm, sweep speed 30 mm/second, high- and low-frequency filters 1–70 Hz, and notch filter 50 Hz, showing bilateral frontotemporal high-amplitude spike and sharp wave discharges.

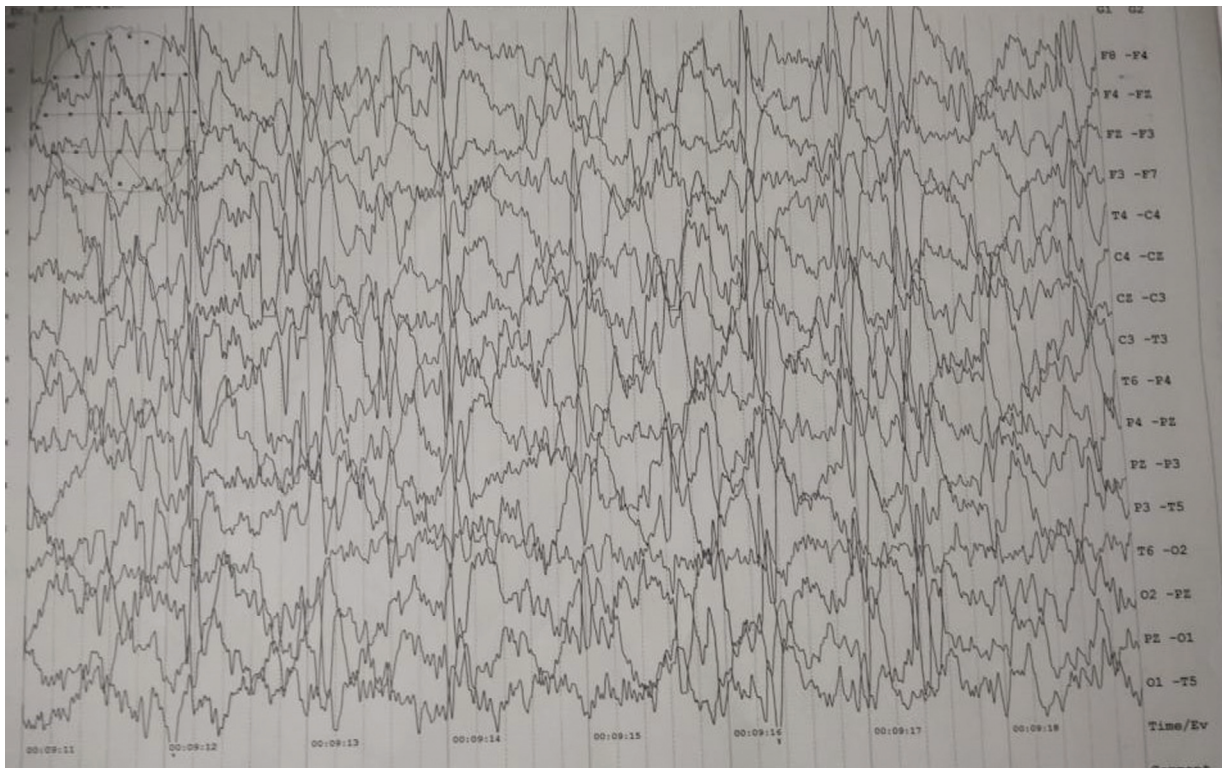


Fig. 5 Bipolar longitudinal montage of sleep EEG at sensitivity 7.5 Hz/mm, sweep speed 30 mm/second, high- and low-frequency filters 1–70 Hz, and notch filter 50 Hz, showing generalized epileptiform discharges.

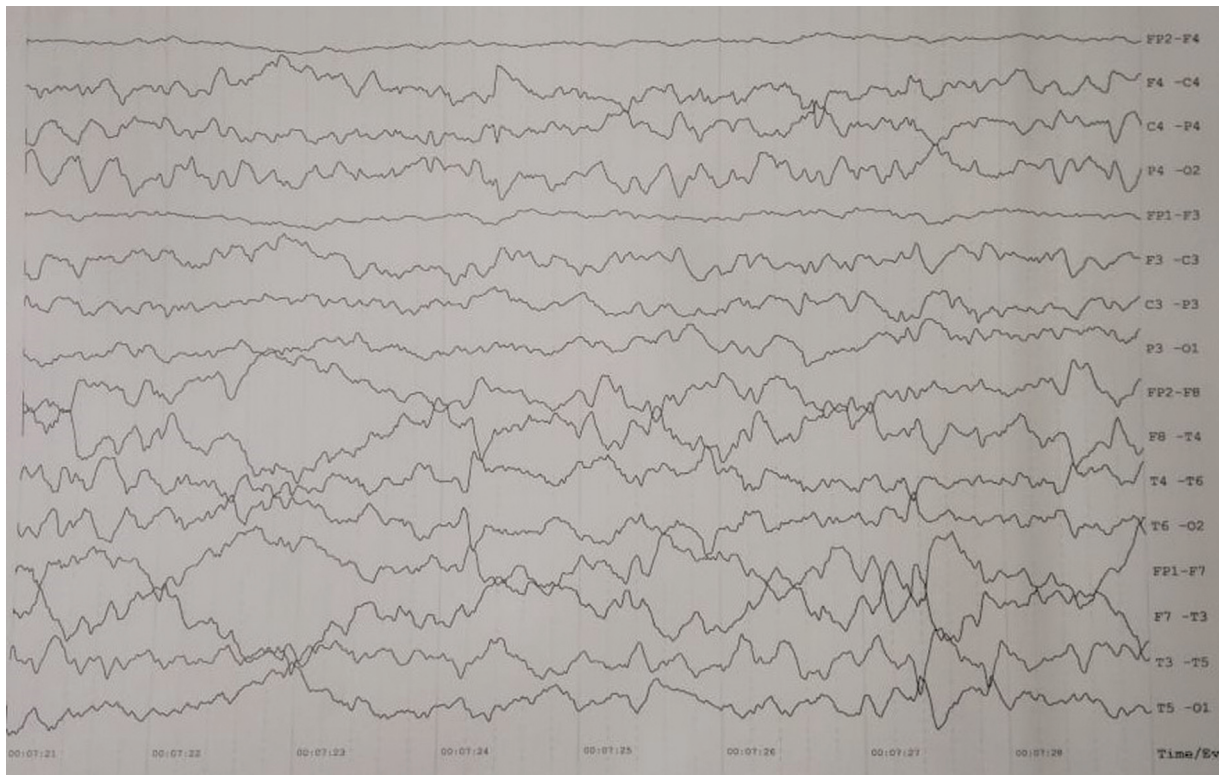


Fig. 6 Bipolar longitudinal montage of sleep EEG at sensitivity 7.5 Hz/mm, sweep speed 30 mm/second, high- and low-frequency filters 1–70 Hz, and notch filter 50 Hz, showing disappearance of the epileptiform discharges, but still lacking the normal sleep changes.

had corpus callosal thinning, delayed myelination, hypomyelination, and periventricular leukomalacia with diffuse cerebral and cerebellar white matter T2 hyperintensities, but subsequent images on follow-up revealed progressive severe generalized cortical and subcortical atrophy with resultant ex vacuo ventricular dilatation—similar to our case, whom we saw late in his disease course.

Microglia are categorized into M1 (classical) and M2 (alternative) phenotypes.⁷ M1 phenotype promotes neurotoxicity by releasing proinflammatory cytokines (tumor necrosis factor alpha, interleukin 6 [IL-6], IL-1 β , IL-12, ROS, nitric oxide, and expression of NOX2), while M2 phenotype induce neuroprotectivity by releasing anti-inflammatory cytokines (IL-10 and TGF- β).⁸ ROS influence phenotypic switching of macrophages.⁹ By α V β 8 integrin-dependent highly localized activation of TGF- β 1 and downregulation of ROS and NOX2, *NRROS* suppress the M1 phenotype, hence promoting neuroprotection.^{1,2,10} *NRROS* deficiency inactivates TGF- β 1 due to loss of its anchorage over phagocytic cells. *NRROS*-deficient mice show presence of reactive microglia, and perivascular macrophages with elevated lysosomal content (indicator of increased phagocytic activity) populate the CNS, thereby promoting autoimmunity.¹¹ Oxidative stress due to ROS modifies and exposes the intraneuronal antigens to the immune system and results in neuroprogression, which is a progressive process of apoptosis, declining neurogenesis, reduced neuronal plasticity, and elevated autoimmune responses.¹²

None of the previously reported cases had associated autoimmune encephalitis triggered by *NRROS* variations.

GABA-B encephalitis is common in the middle-aged and elderly males,¹³ with only two pediatric cases reported till date,^{14,15} the youngest child being 3 years old. The clinical picture of refractory multiple seizure types with rapid global neuroregression signified severe encephalopathy in our case; hence, we chose combined first-line therapy with steroids and IVIG to treat his autoimmune encephalitis.¹⁶ However, the extreme rarity of the entity in infancy and the absence of the characteristic MRI features of limbic encephalitis (temporal lobe, insular and hippocampal T2/fluid-attenuated inversion recovery hyperintense signal changes)¹⁵ prompted us to screen for other (genetic) causes of rapid neuroregression. The resolution of the seizures and disappearance of the cerebral dysrhythmia with immunotherapy pointed toward the suppression of the CNS hyperimmune state promoted by the *NRROS* variation and the autoimmunity triggered by the genetic variation. IVIG induce microglial switch towards protective polarisation, and the ablation of these microglia led to the loss of neuroprotective effects of IVIG. Microglia stimulated by IVIG exhibited reduced neuronal apoptosis and death when exposed to glucose deprivation and ROS *in vitro*.¹⁷ Although none of the previous authors have described the treatment modality, based on our experience with the index case and the above-mentioned experimental data, we propose immunomodulation as a possible approach to treat SENEBC. Probably, earlier immunomodulation would have resulted in better functional outcome in our case. Viewing the CNS proinflammatory state in SENEBC, immunomodulation would likely be effective in cases even without associated autoimmune encephalitis.

Table 1 Showing the different reported variations of the *NRROS* gene

Author	Genetic variations	Type of variation	Zygosity
Smith et al ²	c.1777C > T/p.(Gln593*)	Nonsense	Homozygous
	c.1257del/p.(Gly420AlafsTer14)	Nonsense	Homozygous
Dong et al ⁴	c.1981delC, p.Leu661Serfs*97	Frameshift	Homozygous
	c.1644delG, p.Thr549Profs*82	Frameshift	Homozygous
	variant 1: c.190delC, p.Leu64Trpfs*81 and variant 2: c.29T > C	Frameshift	Double heterozygous
	p.Leu10Pro	Missense	
Macintosh et al ⁶	c.185T > C, p.Leu62Pro	Missense	Double heterozygous
	c.310C > T, p.Gln104Ter	Nonsense	
Index case	c.1487G > A (p.Trp496Ter)	Nonsense	Homozygous

In our patient, the novel biallelic homozygous NM_198565.3(*NRROS*):c.1487G > A (p.Trp496Ter) variant is predicted to cause protein truncation at 496th amino acid instead of normal 692 amino acids with resultant >10% loss of normal protein function, and it has not been reported previously in gnomAD and 1kG. Sanger sequencing validated both the parents as the heterozygous carriers of the variation. ►Table 1 gives a comprehensive account of the described variations of *NRROS* gene.^{2,4,6}

What Does This Article Add?

- First report of autoimmune encephalitis associated with SENEBC, which was hitherto reported only in mouse experiments.
- First to propose immunomodulation as a possible therapy for SENEBC.
- Adds another novel *NRROS* variation to the recently evolving genetic conundrum of SENEBC.

Conflict of Interest
None declared.

References

- 1 Prinz M, Masuda T, Wheeler MA, Quintana FJ. Microglia and central nervous system-associated macrophages-from origin to disease modulation. *Annu Rev Immunol* 2021;39:251–277
- 2 Smith C, McColl BW, Patir A, et al. Biallelic mutations in *NRROS* cause an early onset lethal microgliopathy. *Acta Neuropathol* 2020;139(05):947–951
- 3 Noubade R, Wong K, Ota N, et al. *NRROS* negatively regulates reactive oxygen species during host defence and autoimmunity. *Nature* 2014;509(7499):235–239
- 4 Dong X, Tan NB, Howell KB, et al. Bi-allelic LoF-*NRROS* variants impairing active TGF-beta-1 delivery cause a severe infantile-onset neurodegenerative condition with intracranial calcification. *Am J Hum Genet* 2020;106(04):559–569
- 5 Leavy O. Inflammation: regulating ROS. *Nat Rev Immunol* 2014;14(06):357
- 6 Macintosh J, Derksen A, Poulin C, et al. Novel biallelic variants in *NRROS* associated with a lethal microgliopathy, brain calcifications, and neurodegeneration. *Neurogenetics* 2022;23(02):151–156
- 7 Zhang J, Buller BA, Zhang ZG, et al. Exosomes derived from bone marrow mesenchymal stromal cells promote remyelination and reduce neuroinflammation in the demyelinating central nervous system. *Exp Neurol* 2022;347:113895
- 8 Colonna M, Butovsky O. Microglia function in the central nervous system during health and neurodegeneration. *Annu Rev Immunol* 2017;35:441–468
- 9 Nguyen HM, Grössinger EM, Horiuchi M, et al. Differential Kv1.3, KCa3.1, and Kir2.1 expression in “classically” and “alternatively” activated microglia. *Glia* 2017;65(01):106–121
- 10 Qin Y, Garrison BS, Ma W, et al. A milieu molecule for TGF-β required for microglia function in the nervous system. *Cell* 2018;174(01):156–171.e16
- 11 Du RH, Sun HB, Hu ZL, Lu M, Ding JH, Hu G. Kir6.1/K-ATP channel modulates microglia phenotypes: implication in Parkinson's disease. *Cell Death Dis* 2018;9(03):404
- 12 Bakunina N, Pariante CM, Zunszain PA. Immune mechanisms linked to depression via oxidative stress and neuroprogression. *Immunology* 2015;144(03):365–373
- 13 Zhu F, Shan W, Lv R, Li Z, Wang Q. Clinical characteristics of anti-GABA-B receptor encephalitis. *Front Neurol* 2020;11:403
- 14 Premakumari NS, Rudrappa S, Patagar PM. Paediatric anti-GABAB receptor encephalitis associated with SARS-CoV-2 (COVID-19) infection. *Int J Contemp Pediatrics* 2021;8:1909–1913
- 15 Kruer MC, Hoeffberger R, Lim KY, et al. Aggressive course in encephalitis with opsoclonus, ataxia, chorea, and seizures: the first pediatric case of γ-aminobutyric acid type B receptor autoimmunity. *JAMA Neurol* 2014;71(05):620–623
- 16 Abound H, Probasco JC, Irani S, et al. Autoimmune Encephalitis Alliance Clinicians Network. Autoimmune encephalitis: proposed best practice recommendations for diagnosis and acute management. *J Neurol Neurosurg Psychiatry* 2021;92(07):757–768
- 17 Häußler V, Daehn T, Rissiek B, et al. Intravenous immunoglobulin (IVIg) induce a protective phenotype in microglia preventing neuronal cell death in ischaemic stroke. *Neuromolecular Med* 2020;22(01):121–132