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Clinical letter

Clinical Letter: A case report of targeted therapy with sirolimus for NPRL3 epilepsy

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1. Introduction

NPRL3 (nitrogen permease regulator-like 3), NPRL2, and DEPDC5 (disheveled, Egl-10, and pleckstrin (DEP) domain-containing 5) form the GATOR1 complex (GTPase-activating protein (GAP) activity toward RAG complex 1) [1]. GATOR1 is in the mammalian target of rapamycin (mTOR) pathway and negatively regulates it (Fig. 1) [2]. Mutations in *DEPDC5*, *NPRL2*, and *NPRL3* are associated with focal epilepsy and cortical malformations [2,3]. Treatment with a mTOR inhibitor reduces seizures in patients with tuberous sclerosis complex, a known mTORopathy [4]. We present our center's experience using a mTOR inhibitor as targeted drug therapy for a patient with a *NPRL3* mutation and intractable epilepsy.

2. Case report

A neonate had muscle twitches within hours of birth. Electroencephalogram (EEG) monitoring showed 4–5 clinical seizures per hour. Semiology included extremity twitching and tongue thrusting. Magnetic resonance imaging (MRI) of the brain was initially interpreted as normal (Fig. 2A). Seizures were intractable to phenobarbital and oxcarbazepine, and he was discharged home with incomplete seizure

control on levetiracetam, phenytoin, and pyridoxine.

At one month old he was readmitted due to seizures; EEG (Fig. 2B) showed development of a burst suppression background along with infantile spasms. He transferred to our hospital at six weeks old, at which time phenytoin and pyridoxine were weaned off, and combination therapy with ACTH at 150 units/m² and vigabatrin at 50 mg/kg/day titrating up to 150 mg/kg/day was started. The brain MRI was reviewed after transfer and showed a large left hemisphere cortical malformation (Fig. 2A). Infantile spasms stopped one week after initiation of ACTH and vigabatrin, and within four weeks the burst suppression EEG background had resolved. EEG at ten weeks old showed frequent left central epileptiform discharges (Fig. 2C). Focal seizures of rhythmic shoulder and/or arm jerking, or head turn with arm extension, were intractable and clonazepam was added. From two to three months old focal seizures continued to worsen, ranging from 20 to 60 per day.

An epilepsy panel (EpiXpanded panel, GeneDx, Gaithersburg, MD) revealed a pathogenic paternally inherited heterozygous deletion within 16p13.3 of 552 kb (16p13.3(93722_646006)), which includes the complete genes for *NPRL3* and *HBA* (hemoglobin subunit alpha) 1 and 2. This deletion was confirmed on microarray. Hematology was consulted as *HBA 1* and *2* are associated with alpha-thalassemia. Globin

Abbreviations: DEPDC5, (disheveled, Egl-10, and pleckstrin (DEP) domain-containing 5); EEG, electroencephalogram; GATOR1 complex, (GTPase-activating protein (GAP) activity toward RAG complex 1); HBA, (hemoglobin subunit alpha); MRI, magnetic resonance imaging; mTOR, mammalian target of rapamycin; NPRL2, (nitrogen permease regulator-like 2); NPRL3, (nitrogen permease regulator-like 3)

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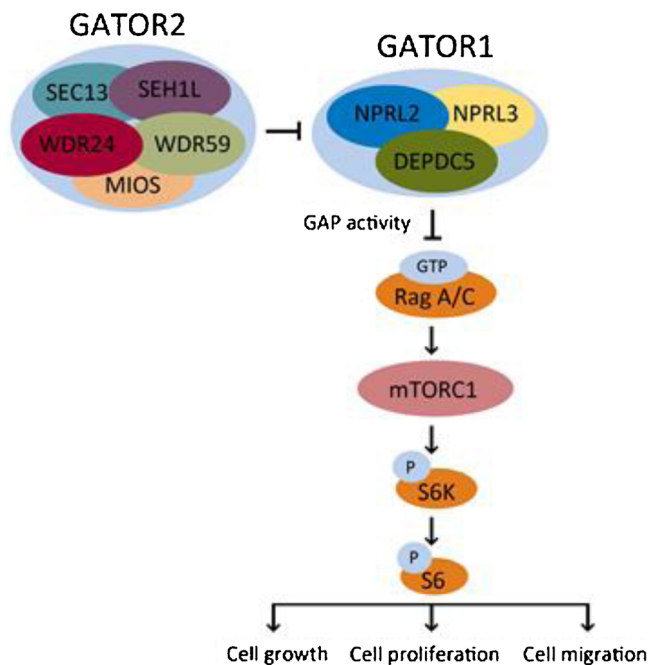


Fig. 1. Schematic representation of the GATOR-mTORC1 pathway. The GATOR1 complex, containing the proteins DEPDC5, NPRL2, and NPRL3, inhibits mTORC1 through its GAP activity toward the GTPases Rag A/C [2].

HBA1/HBA2 testing showed a cis deletion ($\alpha\alpha/-$) consistent with alpha thalassemia trait. The patient is expected to have mild microcytic anemia, which will likely require no further evaluation or treatment.

The infant’s father had spells of unclear etiology as a child, never

confirmed to be seizures. After his son’s mutation was found he was referred to a neurologist. The father’s brain MRI and EEG were normal. Family history was notable for a paternal cousin with febrile seizures, and a paternal cousin once removed with childhood onset epilepsy.

At 3.5 months old the infant was started on sirolimus at 0.8 mg/m² three days per week. Parents were aware that this was off-label usage. After one week seizures began to improve with several seizure free days each week. A sirolimus level two weeks after initiation was 1.5 ng/mL (goal range 5–15 ng/mL); sirolimus was increased to 0.8 mg/m² daily. Three weeks after initiation clinical seizures stopped. Sirolimus level one week later (four weeks after initiation) was 1.8 ng/mL. Though this was below goal range, the patient was clinically seizure free so the dose was not changed. EEG at six months old (Fig. 2D) showed focal slowing, decreased number of focal epileptiform discharges, and no seizures. Throughout the first two months of sirolimus usage the infant had intermittent diaper rashes, eczema, or upper respiratory infections that necessitated holding sirolimus for 3–5 days at a time. At seven months old sirolimus was stopped due to a respiratory infection, and one week later seizures reoccurred. Despite restarting sirolimus seizures continued. Over the next three weeks he had more respiratory infections and an eczema flare, and the decision was made sirolimus couldn’t be tolerated longer. Of note, sirolimus was unable to be titrated past 0.8 mg/m² daily, and a level within goal range was not achieved. Felbamate was tried with no improvement, and at nine months old a left functional hemispherotomy was performed. Pathology showed blurring of the gray-white junction and heterotopic and clustered neurons. Resected tissue demonstrated strong positivity to pS6 stain (Supplementary Fig. 1), consistent with increased mTOR activity.

Post surgery he had right hemisphere seizures that self-resolved. He was then seizure free for six months until seizures occurred during a febrile illness. He has since been seizure free for thirteen additional months.

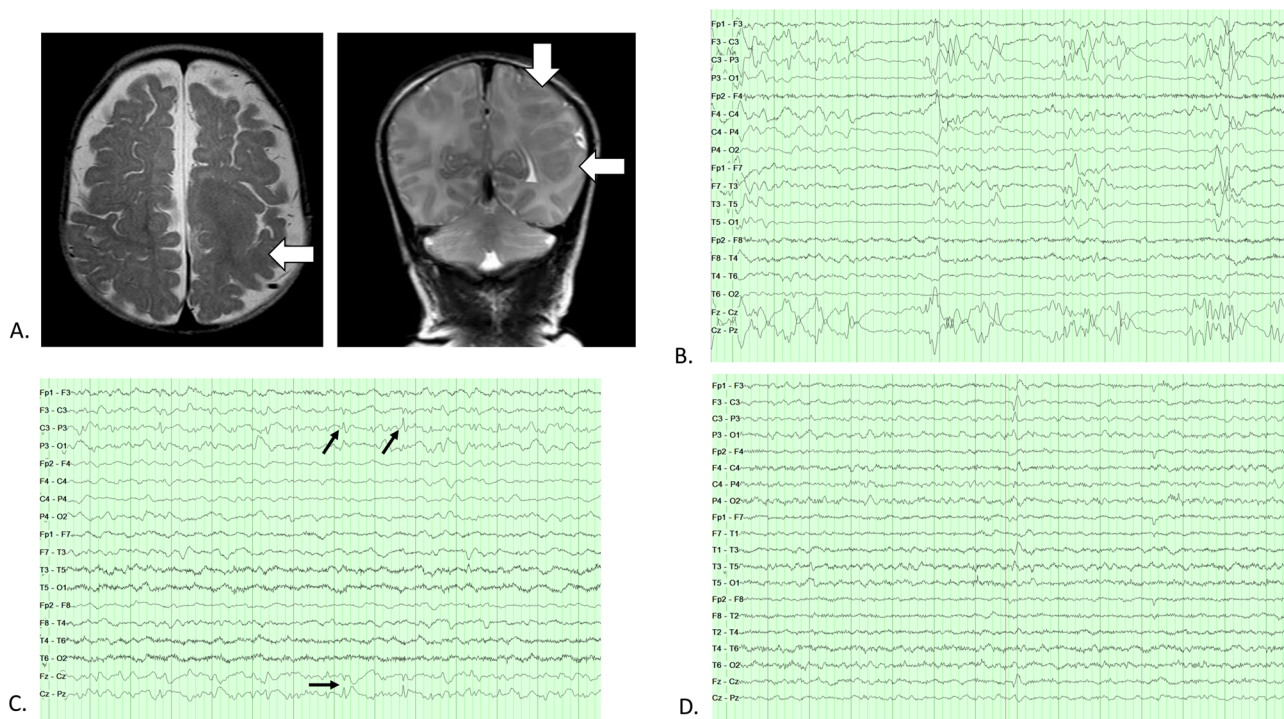


Fig. 2. Brain Imaging and Serial EEGs. A. Brain MRI T2 axial and coronal images at 1 week of life showed thickening and blurring of the gray-white junctions in the left superior frontal, anterior parietal (white arrow), and posterior temporal lobes (white arrow). B. EEG at six weeks old during wakefulness with standard 10–20 placement, bipolar montage, and 10 u V sensitivity, showing burst-suppression pattern with chaotic background. C. EEG at 10 weeks old during wakefulness after treatment with ACTH and vigabatrin, showing resolution of burst-suppression pattern and normal continuous 1–2 Hz posterior dominant background. Also shown are frequent epileptiform discharges in the left central head region (examples of discharges are indicated by black arrows). D. EEG at six months old during wakefulness after treatment with sirolimus, showing a continuous background and reduction in epileptiform discharges.

3. Discussion

Unregulated or hyperactivated mTOR is known to lead to abnormalities in cell growth, neuronal migration, and dendritic spine morphology. We believe this patient's deletion of *NPRL3* resulted in excessive mTOR activation, causing the cortical malformation and intractable epilepsy. We hypothesized that treating with a mTOR inhibitor would improve seizure control. Although sirolimus provided only temporary seizure control for 3.5 months, during that time it completely controlled the infant's epilepsy, allowing him time to grow prior to undergoing epilepsy surgery.

To our knowledge this is the first report of treatment with a mTOR inhibitor for *NPRL3*-related epilepsy. mTOR inhibitors should be considered as treatment options in patients with GATOR mutations. The most common side effects of mTOR inhibitors are stomatitis and respiratory infections. mTOR inhibitors are generally well tolerated, though our patient failed it due to side effects, and have documented effectiveness in epilepsy associated with tuberous sclerosis [4]. A limitation of our case is that it involves a single patient. In addition, precision treatment with sirolimus is limited by it inhibiting the mTOR pathway broadly (leading to many of its side effects), rather than it having focused central nervous system inhibition. Although not a limitation of sirolimus usage, this patient's outcome after epilepsy surgery is supportive evidence that when there is an identifiable epileptic foci, epilepsy surgery is often the most effective treatment.

Although our patient ultimately failed sirolimus due to side effects, initial results were very encouraging. Even if a patient is unable to tolerate a mTOR inhibitor long-term, temporary seizure control can be beneficial until other treatment options are suitable.

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Declaration of Competing Interest

MVL, CF, and HG have no competing interest. DNF has received honoraria, travel support, and his employer has received research funding from Novartis Pharmaceuticals. Fig. 1 was previously published and is included in this paper with permission from the original article's publisher.

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We are grateful to the family for allowing us to publish their child's history and experience. Family gave informed consent for the patient's history, EEG, and MRI to be published.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.seizure.2019.10.007>.

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