■ Clinical commentary

Epileptic Disord 2021; 23 (5): 739-743

Epileptic Disorders

Beneficial effects of the ketogenic diet on drug-resistant epileptic encephalopathy associated with a *de novo NBEA* pathogenic variant

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Received June 23, 2020; Accepted April 06, 2021

ABSTRACT

Although neurobeachin (*NBEA*) *de novo* genetic variants have been mainly reported in patients with neurodevelopmental disorders (NDD), they have also been recently associated with early childhood epilepsy. We report an 11-year-old boy who was first evaluated at 34 months of age because of drug-resistant epileptic encephalopathy. He also had developmental delay and prominent autistic features. Whole-exome sequencing (WES) disclosed a pathogenic *NBEA* c.5258_5279del, p.(Ala1753Valfs*13) variant, occurring *de novo* and a paternally-inherited heterozygous *NBEA* c.416T>C p.(Met139Thr) variant of uncertain significance (VUS). The patient showed good response to the ketogenic diet, suggesting that this therapy may be an effective option for patients with seizures who carry *NBEA* variants.

Key words: drug-resistant epilepsy, epileptic encephalopathy, neurodevelopmental disorder, ketogenic diet, *NBEA*

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doi:10.1684/epd.2021.1327

NBEA (neurobeachin) gene, mapping to chromosome 13q13 [1], encodes neurobeachin, a member of various groups of A-kinase anchor proteins involved in neuronal post-Golgi membrane trafficking, dendritic spine formation and synaptic function, predominantly expressed in the brain during development [2-5]. Microdeletions and reciprocal balanced translocations involving the *NBEA* gene have been documented in patients with autistic spectrum disorder (ASD) and prominent autistic features [6-9]. Subsequent studies identified two *de novo* variants (one loss-of-function and one missense variant, predicted to be benign) in ASD probands from the Simons Simplex Collection and Autism Sequencing Consortium [10, 11]. *NBEA* has also been suggested to be a developmental disorder (intellectual disability and/or developmental delay)-related gene, based on the identification of two *de novo* variants by whole-exome sequencing (WES) [12]. The potential role of *NBEA* in autism and other neurodevelopmental disorders (NDD) has been further supported by functional studies in mice and Drosophila [13, 14].

Recently, Mulhern *et al*. reported 24 *de novo NBEA* variants in a series of patients with NDD; epilepsy in the majority and autism or prominent

autistic features in half of the cohort. Additionally reported clinical findings were behavioural problems, abnormal movements, hypotonia and microcephaly [15]. Of those with epilepsy, most had early childhood-onset generalized epilepsy or mixed (focal and generalized) epilepsy with multiple seizure types, among which myoclonic seizures (with onset mainly between one and four years of age) were most common, especially in patients with loss of function variants, who frequently showed a myoclonic-atonic epilepsy (MAE)-like phenotype [15]. Response to treatment was variable; in some patients, seizure freedom was achieved between three and 19 years of age, either on valproic acid, ethosuximide, levetiracetam, lamotrigine, benzodiazepines or dietary therapy, mainly in combination. Other patients proved refractory to treatment [15].

We present the clinical, genetic and treatment data of a patient recently diagnosed with drug-resistant epileptic encephalopathy and intellectual disability, associated with two heterozygous biallelic *NBEA* variants, who showed good response to a ketogenic diet.

Case study

A male patient, now aged 11 years, was born at term after uneventful pregnancy and delivery (normal Apgar scores and birth weight), from non-consanguineous Caucasian parents. His father reportedly had febrile seizures as a toddler and has possible intellectual disability (not tested). His early developmental milestones were delayed (independent walking and first words between 18 and 24 months). Urinary and faecal continence were never achieved. Sporadic falls due to clumsiness were reported.

He was first evaluated at our child neurology unit at 34 months of age because of two febrile seizures and a cluster of afebrile seizures, occurring in the previous two weeks. After a few days, he was admitted to hospital due to relapsing epileptic seizures. He developed a severe epileptic encephalopathy characterized by multiple seizure types, including generalized motor (bilateral tonic-clonic, myoclonic), focal impaired awareness with non-motor onset (behaviour arrest) progressing to chewing automatisms, and focal motor onset (clonic) with retained awareness, involving the upper limbs with right predominance. Some of the seizures were associated with autonomic features (oxygen desaturation). Episodes seemed to be triggered by sudden light changes, and they occurred at any time of the day, predominantly during wakefulness. Clusters were usually characterized by 7-8 episodes per hour.

At onset, only intramuscular depot injection of adrenocorticotropin hormone (ACTH) combined with repeated intravenous high-dose midazolam boluses led to partial seizure control, but episodes recurred at any attempt to taper off midazolam.

During follow-up, pharmacological approaches included: valproic acid, topiramate, phenytoin, levetiracetam, midazolam, diazepam, clonazepam, nitrazepam, clobazam, pyridoxine and corticosteroids (methylprednisolone, deflazacort), usually in combination. Some drugs resulted in limited or non-significant clinical benefits, while other medications were withdrawn because of side effects (erratic myoclonus with phenytoin and prominent psychomotor agitation on nitrazepam and clobazam).

Given the lack of seizure control with antiepileptic drugs (at the time he was on levetiracetam, topiramate and clonazepam), a 3:1 ketogenic diet was started. This approach led to progressive seizure freedom, allowing for antiepileptic drug withdrawal after about one year.

Aged six years, moderate intellectual disability was diagnosed, associated with speech disorder and prominent autistic features, such as impaired social relationships, stereotypies and echolalia.

At nine years of age, he was admitted to hospital again due to recurrence of generalized non-motor (absence) seizures occurring multiple times per day, more frequently in the morning. Laboratory investigations revealed a lack of ketosis. After re-inducing ketosis during the hospital stay, we documented restoration of the beneficial effect of the 3:1 ketogenic diet within a few days. During follow-up, in the following year, the parents reported periods characterized by one or two absence episodes per week. Currently, he is on the ketogenic diet with satisfactory seizure control.

At the latest follow-up visit (10 years age), his clumsiness had become more prominent, while of his occipito-frontal circumference (OFC) and the rest of his neurological examination were confirmed to be normal.

Serial electroencephalograms (EEG) during the first hospital admission showed diffuse excess of slow activity, but no clear-cut interictal discharges. On one occasion, an electroclinical seizure was recorded, consisting of a 20-second ictal discharge of slow waves with irregularly intermixed spikes, clinically accompanied by bilateral tonic-clonic jerking, followed by transient EEG depression. EEG in the post-ketogenic diet phase showed abnormal background activity with no clear epileptiform discharges.

At the latest follow-up visit (10 years), EEG showed focal spike-wave complexes over the left fronto-centro-temporal regions.

Neurometabolic investigation revealed normal results: plasma and cerebro-spinal fluid amino acids, acylcarnitines and urinary organic acids profiles, and cerebrospinal fluid examination (lactate, herpes virus, glycorrhachia). Screening for celiac disease was also negative.

Brain MRI did not show any specific features (the most recent was performed at 10 years of age). Abdomen ultrasound was normal. A muscle biopsy with enzymatic analysis of the respiratory chain and pyruvate dehydrogenase disclosed reduced pyruvate dehydrogenase levels and normal activity in cultured fibroblasts. No variants in the genes encoding the six subunits (*PDHA1*, *PDHB*, *DLAT*, *PDHX*, *DLD* and *PDP1*) of the pyruvate dehydrogenase complex were documented by direct sequencing or by WES plus copy number variation analysis.

Direct sequencing of the *SLC2A1*, *SCN1A*, *SCN1B*, *ARX*, *CDKL5*, *FOXG1* and *PCDH19* genes also revealed no variants. Array-CGH was normal.

WES revealed a c.2257C>Tp.(Pro753Ser) heterozygous missense variant in the *HCN1* (hyperpolarization activated cyclic nucleotide gated potassium channel 1) gene, inherited from the father, which was classified as "likely benign" according to the American College of Medical Genetics and Genomics (ACMG) guidelines [16]. WES also identified a *de novo* c.5258_5279del, p.(Ala1753Valfs*13) heterozygous variant and a paternally-inherited c.416T>C p.(Met139Thr) heterozygous variant in the *NBEA* gene. No additional variants were detected.

Discussion and conclusions

To the best of our knowledge, the *NBEA* gene variants we found in our patient have not been previously reported in the medical literature and were not observed in large reference population cohorts of the Genome Aggregation Database. Diseases caused by *NBEA* variants are inherited in an autosomal dominant fashion. The paternally-inherited c.416T>C p.(Met139Thr) missense variant was classified as a variant of "uncertain significance" (VUS) according to the ACMG criteria [16], as there was insufficient evidence to evaluate its clinical relevance. On the contrary, the c.5258_5279del, p.(Ala1753Valfs*13) variant is a 23-bp deletion which is predicted to cause a transitional frameshift, leading to a premature stop codon, 12 residues downstream in the new reading frame, thus predicted to cause loss of normal protein function. Based on an association between the gene and our patient's phenotype, its absence in control populations and the type of variant type, this variant was therefore classified as pathogenic [16]. In fact, *NBEA* is extremely intolerant to loss-of-function variation [17, 18].

NBEA loss-of-function variants have been reported in the literature in individuals with ASD and developmental delay/intellectual disability [2, 12], as well as in subjects with schizophrenia and obesity [19, 20]. Other *NBEA de novo* missense variants are reported from various NDD cohorts, but none were shown to be significant, and none of the individuals were specifically reported to have epilepsy [10, 21, 22].

Although *NBEA* has previously been prioritized as a strong candidate gene for epileptic encephalopathies based on a high level of co-expression with known epileptic encephalopathy genes [23-25], an association with epilepsy was first reported by Monlog and coworkers, who identified a partial deletion of *NBEA* in an individual with idiopathic generalized epilepsy [26]. Mulhern *et al*. recently described 24 *de novo NBEA* variants in patients with NDD and epilepsy, an association which had not been described in previous reports regarding the *NBEA* gene [15]. We previously described other gene variants associated with developmental and epileptic disorders [27-30], however, none of these affected the *NBEA* gene.

The *NBEA* gene appears to be involved in synaptic structure and function. Importantly, a growing body of evidence implicates genes encoding synaptic proteins in NDD associated with epilepsy, autism, or intellectual disability [15]. The observed comorbidity between these nosologically distinct phenotypes is determined by overlapping causative genes, which again reflects the involvement of similar molecular processes in their pathogenesis [31].

In agreement with the observations of Mulhern *et al*. [15], we reported a case of a *de novo NBEA* variant in a child with NDD and epileptic encephalopathy with onset before three years of age, characterized by refractory polymorphous seizures. The patient clearly benefited from a 3:1 ketogenic diet, allowing initial seizure freedom and withdrawal of all combined antiepileptic drugs, one year after it was started. Mulhern and colleagues mentioned "some success" with dietary therapy in some of their patients, but this finding was not further specified [15]. Therefore, we would suggest considering the ketogenic diet early on for these patients, in agreement with the most recent guidelines on dietary therapies [32].

Although pyruvate dehydrogenase deficiency can cause early-onset epilepsy with a very good response to the ketogenic diet, and its presentation can be heterogeneous (from fatal lactic acidosis to relapsing ataxia), our patient lacked many of the typical features found in patients harbouring pyruvate dehydrogenase complex deficiency, such as lactic acidosis, dysmorphic features, central nervous system malformations or basal ganglia involvement [33] and no pathogenic/likely pathogenic variants of causative genes were disclosed.

The classic ketogenic diet (CKD) consists of a high-fat (90%) and low-protein and carbohydrate diet, introduced to produce ketosis and simulate a starvation state. Our understanding of its mechanisms of action is still incomplete, however, modulation of neuronal

metabolism and excitability resulting in reduced seizure frequency have been proposed [34]. Various studies found a significantly positive outcome for treatment of refractory epilepsy in children [35, 36]. Based on our report, a 3:1 ketogenic diet may be an option for treatment of intractable epilepsy associated with *NBEA* gene variants. ■

Disclosures.
None of the authors have any conflict of interest to disclose.

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TEST YOURSELF

(1) What clinical features have been described in the literature in association with *NBEA* variants?

(2) What are the beneficial effects of the ketogenic diet?

Note: Reading the manuscript provides an answer to all questions. Correct answers may be accessed on the website, www.epilepticdisorders.com.