NBEA

CAUSES Study; EuroEPINOMICS-RES-MAE Working Grp; Mulhern, Maureen S.; Stumpel, Constance; Stong, Nicholas; Brunner, Han G.; Bier, Louise; Lippa, Natalie; Riviello, James; Rouhl, Rob P. W.

Published in:
Annals of Neurology

DOI:
10.1002/ana.25350

Publication date:
2018

NBEA: Developmental Disease Gene with Early Generalized Epilepsy Phenotypes

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Received Apr 26, 2018, and in revised form Aug 27, 2018. Accepted for publication Sep 23, 2018

View this article online at wileyonlinelibrary.com. DOI: 10.1002/ana.25350.

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NBEA is a candidate gene for autism, and de novo variants have been reported in neurodevelopmental disease (NDD) cohorts. However, *NBEA* has not been rigorously evaluated as a disease gene, and associated phenotypes have not been delineated. We identified 24 de novo *NBEA* variants in patients with NDD, establishing *NBEA* as an NDD gene. Most patients had epilepsy with onset in the first few years of life, often characterized by generalized seizure types, including myoclonic and atonic seizures. Our data show a broader phenotypic spectrum than previously described, including a myoclonic-astatic epilepsy–like phenotype in a subset of patients.

ANN NEUROL 2018;84:796–803

NBEA encodes neurobeachin, a brain-specific kinase-anchoring protein implicated in vesicle trafficking and synaptic structure and function.[1] It is not currently associated with disease in the Online Mendelian Inheritance in Man (OMIM) database but is a candidate gene for autism based on linkage studies and the identification of microdeletions and a reciprocal balanced translocation involving *NBEA* in patients with autism.[2–6] More recently NBEA has been suggested to be a neurodevelopmental disease (NDD) candidate gene based on the observation of two de novo variants identified by whole exome sequencing (WES).[7]

Here, we report 24 de novo *NBEA* variants, including 18 identified through clinical and research sequencing and 6 de novo deletions identified by array or whole genome sequencing (WGS). De novo *NBEA* mutations were associated with NDD in all patients and epilepsy in the majority. Of those with epilepsy, most had generalized seizure types and seizure onset in the first few years of life. A subset had features of myoclonic-astatic epilepsy (MAE), with toddler age onset of myoclonic, atonic, and/or myoclonic-atonic seizures and, in some cases, developmental regression with seizure onset. Epilepsy has not been described in previous reports implicating *NBEA* as an autism candidate gene, but our data support that it is a major feature in patients with variants in *NBEA*.

Subjects and Methods

NBEA variants were identified by research trio WES for Individuals 1, 6, 10, 14, and 18 and research trio WGS for Individuals 13 and 24. Individuals 11 and 12 and their parents underwent sequencing of a panel of candidate genes based on a previous sequencing project. Variants identified in Individuals 1, 10, 12, 13, 14, and 18 were confirmed by Sanger sequencing. For Individual 24, the deletion was confirmed by research global screening array. For Individual 19, the deletion was identified through a research protocol using single nucleotide polymorphism array. *NBEA* variants were identified in Individuals 2 to 5, 7 to 9, and 15 to 17 through clinical WES, and were either Sanger confirmed or met laboratory criteria for reporting based on WES only. Individuals 20 to 23 and their parents underwent clinical microarray. Informed consent was obtained by parents, and all research studies received prior approval by the appropriate institutional review board. Groups were connected through GeneMatcher.[8]

Results

NBEA variants were identified in 22 previously unreported cases and 2 cases already reported in the literature.[7] All variants were confirmed to be de novo with the exception of Individual 13. For Individual 13, parents were unavailable for Sanger confirmation, but we presumed (based on demographic information and referral site) that this was the same person included in an epileptic encephalopathy cohort, where the variant was confirmed to be de novo.[9,10] Variant types identified included nonsense (8/24), frameshift (5/24), missense (4/24), intragenic deletion (5/24), splice site (1/24), and a multigene deletion (1/24). All variants were absent from gnomAD and from 12,325 internal Institute for Genomic Medicine controls.[11] Each of the missense variants were predicted to be deleterious by at least 1 computational model; of the 4 missense variants, 3 were predicted probably damaging and 1 possibly damaging (Individual 17) by PolyPhen-2 and all had an adjusted Combined Annotation Dependent Depletion score ≥ 20.[12] Two were found within WD-40 repeats (Fig 1). *NBEA* encodes 2 CCDS transcripts—the 2,946 amino acid CCDS45026/NM_015678 (NP_056493.3) and the shorter 739 amino acid CCDS55894/NM_001204197 (NP_001191126.1). All variants identified in our cohort affected the longer transcript, and 8 affected both. Nonsense and frameshift variants from our cohort occurred throughout the protein (NP_056493.3). All deletions were intragenic, except for the 2.87Mb deletion identified in Individual 19, which contains multiple other genes, none of which is known to be associated with disease based on OMIM.

All patients had NDD, including developmental delay (DD) and/or intellectual disability (ID) (see Table 1). Age of ambulation ranged from 11 months to 3.5 years; 1 person (Individual 11) was nonambulatory as an adult. All patients had some level of speech delay, and 2 patients were nonverbal at 11 and 19 years (Table 2). Two patients had developmental regression noted at the time of seizure onset. Half of the patients (12/24) had autism or prominent autistic features.

The majority of patients (15/24) had epilepsy, and 2 additional patients had presumed or possible epilepsy. Of those with classified epilepsy, one had focal epilepsy; the rest had generalized epilepsy or mixed (focal and generalized) epilepsy with multiple seizure types. Myoclonic seizures were common, present in almost half of those with epilepsy (7/15). Of these, 4 presented with an MAE-like phenotype, with toddler onset atonic and/or myoclonic-atonic seizures. Myoclonic seizures were more common in patients with loss-of-function (LoF) variants (6/12, 50%) than those with missense variants (1/3, 33.3%), and all cases of atonic and/or myoclonic-atonic seizures (n = 5) were found in individuals with an LoF mutation.

About three-quarters of patients with epilepsy had seizure onset between 1 and 4 years of age. The 2 individuals with seizure onset at <1 year of age both had LoF variants. Response to treatment was variable; some patients had cessation of seizures between the ages of 3 and 19 years, whereas others remained refractory to treatment. Some success was reported using valproic acid, ethosuximide (with valproic acid), levetiracetam, lamotrigine,

TABLE 1. Summary of Clinical and Epilepsy Characteristics by Variant Type

Clinical Characteristic	All Variants, n = 24	LoF Variants, n = 20[a]	Missense Variants, n = 4
Neurodevelopmental disability	24 (100%)	20 (100%)	4 (100%)
Developmental regression	2 (8.33%)	2 (10%)	0
Autistic features or autism	12 (50%)	10 (50%)	2 (50%)
Microcephaly or borderline microcephaly	4 (16.67%)	4 (20%)	0
Epilepsy	15 (62.50%)	12 (60%)	3 (75%)
Epilepsy Characteristic	**All Variants, n = 15**	**LoF Variants, n = 12**	**Missense Variants, n = 3**
Age of epilepsy onset			
<1 year	2 (13.33%)	2 (16.67%)	0
1–4 years	11 (73.33%)	8 (66.67%)	3 (100%)
>4 years	2 (13.33%)	2 (16.67%)	0
Generalized seizures	12 (80%)	10 (83.33%)	2 (66.67%)
Myoclonic	7 (46.67%)	6 (50%)	1 (33.33%)
Atonic and/or myoclonic-atonic	5 (33.33%)	5 (41.67%)	0
Absence or atypical absence	5 (33.33%)	5 (41.67%)	0
Tonic, clonic, and/or tonic-clonic	10 (66.67%)	8 (66.67%)	2 (66.67%)
Focal and generalized seizures	4 (26.67%)	2 (16.67%)	2 (66.67%)
Focal seizures only	1 (6.67%)	0	1 (33.33%)
Unclassified seizures only	2 (13.33%)	2 (16.67%)	0
Epileptiform abnormalities on EEG			
Generalized spike/polyspike and wave	9 (60%)	8 (66.67%)	1 (33.33%)
Focal and generalized discharges	1 (6.67%)	1 (8.33%)	0
Focal discharges	2 (13.33%)	1 (8.33%)	1 (33.33%)
Unclassified discharges	2 (13.33%)	2 (16.67%)	0
No epileptiform abnormalities	1 (6.67%)	0	1 (33.33%)

[a]All deletions, frameshift, nonsense, and splice-site variants were considered LoF for this table.
EEG = electroencephalogram; LoF = loss of function.

TABLE 2. Genotype and Phenotype Details for Individuals with *NBEA* Variants

Individual	Variant (NM_015678.4, hg19); Variant Type	Sex/ Age, yr	Development	Autism	Seizure Types	Age at 1st Seizure
1	c.1006C>T; p.Arg336*; NS	M/6	No motor delay; mild speech delay	–	Myoclonic, astatic, absence, myoclonic-clonic-tonic, GTC, tonic, focal unaware, SE	3 yr
2	c.6829C>T; p.Arg2277*; NS	F/21	W = 18 mo; NV	++	Tonic, GTC	3 yr
3	c.3994C>T; p.Pro1332Ser; MS	M/4	W = 30 mo; speaks some 2-word phrases	+	None	N/A
4	c.4484del; p.Asn1495Ilefs*17; FS	M/19	W = 2.5 yr; speaks words at 16 yr	++	Unknown	3.5–4 yr
5	c.6313G>T; p.Glu2105*; NS	M/18	W = 15–17 mo; slightly delayed speech; regression at 2 yr	–	Myoclonic, atonic, atypical absence, clonic, GTC	2 yr
6	c.7294_7295dup; p.Glu2433Argfs*3; FS	F/13	W = prior to 15 mo; first word = prior to 2 yr	–	None	N/A
7	c.7707 + 2T>C; SS	F/18	W unavailable; speech delay	–	Febrile, absence	<1 yr
8	c.6868C>T; p.Gln2290*; NS	M/3	W = 19 mo; speaks < 10 words at 3 yr	+	None	N/A
9	c.7462G>T; p.Glu2488*; NS	F/19	W = 12 mo; speech delay	–	None	N/A
10	c.7230del; p.Asp2411Ilefs*21; FS	M/9	W = 11 mo; first word = 16 mo	++	Myoclonic, myoclonic-atonic, atypical absence, GTC	19 mo
11	c.3183delA; p.Glu1062Argfs*8; FS	M/19	Nonambulatory; NV	+	Myoclonic, myoclonic-atonic, GTC, focal unaware, SE	19 mo
12	c.1448C>T; p.Ala483Val; MS	F/11	W = 15 mo; first word = 30 mo	–	Febrile, hemiconvulsive, GTC	1 yr
13	c.6637C>T; p.Arg2213*; NS	F/23	Normal until regression at 26 mo	–	Atonic, tonic, GTC	26 mo
14	c.2836C>T; p.His946Tyr; MS	F/20	W = 18 mo; words only (no phrases) at 2 yr	–	Nocturnal frontal lobe seizures	2 yr
15	c.3832C>T; p.Arg1278*; NS	F/5	W = 1 yr; severe speech delay	–	None	N/A
16	c.3362del; p.Asn1121Metfs*9; FS	F/24	W = 2 yr; first word at 2 yr	++	Generalized	19 yr
17	c.8401G>A; p.Glu2801Lys; MS	M/11	W = 15 mo; 10 words at 24 m	+	Myoclonic, tonic, focal, focal to GTC, febrile	14 mo

TABLE 2. Continued

Individual	Variant (NM_015678.4, hg19); Variant Type	Sex/ Age, yr	Development	Autism	Seizure Types	Age at 1st Seizure
18	c.4715C>A; p.Ser1572*; NS	F/3	W = 14 mo; 10–15 words at 2 yr	+	None (paroxysmal spells not confirmed to be seizures)	N/A
19	chr13:33957317-36828237 x1; MGD	M/15	W = 3.5 yr; first word at 14 mo; stagnation until 4 yr	–	Myoclonic, GTC, epileptic spasms/tonic	18 mo
20	chr13:35590335-35940429 x1; IGD	M/16	W = 15 mo; first word = 3 yr	–	None	N/A
21	chr13:35574513-36163037 x1; IGD	M/16	W = 2 yr; NV at 11 yr	+	None	N/A
22	chr13:36038249-36141224 x1; IGD	M/11	W unavailable; IQ = ~60	+	Myoclonic, GTC, tonic	8 mo
23	chr13:35963197-36125577 x1; IGD	M/5	W = 18 mo; delayed speech	+	Possible absences	4 yr
24	chr13:35700830-35887000 x1; IGD	F/9	W = 17 mo; first word at 18–20 mo	–	Nonconvulsive SE, generalized	2 yr

+ = autistic features; + + = autism diagnosis; F = female; FS = frameshift; GTC = generalized tonic-clonic; IGD = intragenic deletion; IQ = intelligence quotient; M = male; MGD = multigene deletion; MS = missense; N/A = not applicable; NS = nonsense; NV = nonverbal; SE = status epilepticus; SS = splice site; W = age at walking.

benzodiazepines, and dietary therapy, usually in some combination (see Table 2). The interictal electroencephalogram (EEG) often showed diffuse slowing and included generalized epileptiform abnormalities in the majority of patients with epilepsy (9/15, 60%). Magnetic resonance imaging did not show any distinctive features in most patients.

Seven participants had behavior problems, including aggression (4/24) and attention deficits and hyperactivity (4/24). Eight patients had abnormal movements, including wide-based, uncoordinated gait (6/24) and dystonic movements (3/24); 1 additional patient was noted to be "clumsy." Other features reported in >1 patient include hypotonia of variable severity (8/24), microcephaly or borderline microcephaly (4/24), recurrent infections (2/24), and eczema or dry skin (3/24). All of those with microcephaly had LoF variants.

Discussion

We have demonstrated that *NBEA* is an NDD gene, associated with early childhood epilepsy, which is consistent with what is known about the biology of *NBEA*. Neurobeachin (NBEA) is a brain-specific multidomain scaffolding protein belonging to the family of BEACH (Beige and Chediak-Higashi) domain-containing proteins, which play a role in vesicle trafficking and dynamics.[1,13] NBEA localizes to vesicular structures at the trans-face of the Golgi apparatus and within neuronal dendrites and appears to regulate synaptic structure and function through targeted trafficking of postsynaptic proteins.[14,15] In zebrafish, postsynaptic NBEA is essential for both electrical and chemical synapse formation and maintenance of dendritic complexity.[16] Our work adds to a growing body of literature implicating genes encoding synaptic proteins in neurodevelopmental disorders associated with epilepsy, autism, and ID, although the specific mechanism by which these variants give rise to NDD is unknown.[17,18]

NBEA has been considered an autism candidate gene, and de novo *NBEA* variants have been seen in NDD cohorts. A large genetic linkage study indicated the 19cM segment on chromosome 13 containing *NBEA* as a candidate region for autism.[2] A patient with sporadic autism was found to have a de novo balanced reciprocal translocation (t[5;13][q12.1;q13.2]) disrupting *NBEA*.[3] Three patients with autism have been described in the literature with monoallelic deletions that include *NBEA*.[4–6] Two individuals with de novo *NBEA* single nucleotide variants (Individuals 13 and 14) were previously described in a

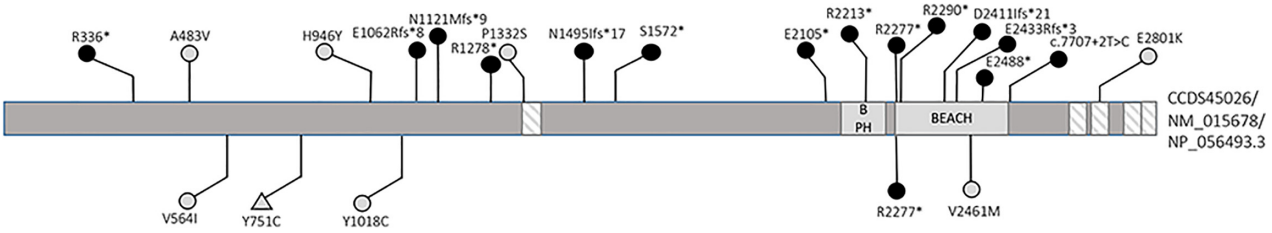

FIGURE 1: Mutational landscape of *NBEA*. Domain organization of the NBEA protein is based on Uniprot. Boxes indicate functional domains; beach type PH (BPH), BEACH domain, and WD-40 repeat domains (lined) are shown. The positions of variants described in our cohort are shown above the protein, and those described in other cohorts are shown below. Black circles represent nonsense, frameshift, and splice site variants, and light circles represent missense variants. All variants identified in a person with a neurodevelopmental disease are represented with a circle, and the variant identified in a control individual is represented with a triangle.

cohort with ID and/or DD.[7] Although *NBEA* has been prioritized as a strong candidate epileptic encephalopathy (EE) gene based on high level of coexpression with known EE genes in the adult and developing brain, an association with epilepsy has not previously been reported.[19]

In reporting 24 patients with NDD harboring de novo variants absent from population databases, we provide substantial genetic evidence implicating *NBEA* in disease. The majority of the variants (20/24) predict LoF, and *NBEA* is extremely intolerant to LoF variation (probability of LoF intolerance = 1, Residual Variation Intolerance Score [ExAC v2] = 1.16%),[11,20] suggesting a haploinsufficiency disease mechanism. Our work complements experimental data demonstrating a role for *NBEA* at the synapse and autism-like behaviors modeled in the $Nbea^{+/-}$ mouse.[21] Applying ClinGen's clinical validity of gene–disease associations framework, we therefore conclude that there is "strong" evidence that pathogenic variants in *NBEA* cause NDD with and without epilepsy.[22]

In terms of the overall phenotype of the cohort, language delay was universal, but the vast majority of patients were ultimately verbal, and almost all patients were ambulatory by 4 years of age; half had autism or autistic features and half had epilepsy within the first years of life, typically a generalized epilepsy often with myoclonic seizures. A subset had an MAE-like phenotype.

MAE is rare, accounting for only 1 to 2% of epilepsy in the first decade of life.[23] Although a genetic etiology was speculated in the first description by Doose, the vast majority of cases of MAE remain genetically unexplained. MAE-like phenotypes have been described in association with variants in *SLC2A1, SCN1A, SCN1B, GABRG2*, and *GABRB3*.[24–26] The core *NBEA*-associated epilepsy in our cohort involves toddler age onset of multiple generalized seizure types, especially myoclonic seizures, with MAE-like phenotypes similar to what has been described for *CHD2* and *SLC6A1*. As with these genes, the phenotypic spectrum of *NBEA* epilepsy includes patients with features atypical for MAE, such as DD preceding seizure onset and the presence of focal epileptiform discharges and/or focal seizures in some patients.[27–29] A limitation of the current study is the lack of standardization in the evaluation of patients. Diagnosis of seizure types and syndromes and EEG interpretation were as determined by the treating neurologists. Future work with centralized review of polygraphic video-EEG for each reported seizure type and a common neuropsychological battery applied universally across the cohort will give a better understanding of the *NBEA* phenotypic spectrum.

Although the small number of, and lack of recurrent, missense variants limit our ability to make definite phenotype–genotype correlations, it is perhaps notable that MAE seizure types, including myoclonic, atonic, and/or myoclonic-atonic seizures, were seen almost exclusively with LoF variants. Additionally, epilepsy with onset in infancy and microcephaly or borderline microcephaly were each only seen with LoF variants, possibly reflecting a more severe phenotypic spectrum in this group.

In addition to the cases from our cohort, 3 other de novo missense variants are reported in denovo-db (http://denovo-db.gs.washington.edu) from various NDD cohorts, although *NBEA* variants were not significant in any of these cohorts. The cohorts included autism spectrum disorders, schizophrenia, and developmental disability/ID, but none of these individuals was specifically reported to have epilepsy or seizures.[30–32] Individual 4 was previously included in an ID cohort, and as mentioned before, Individual 13 was probably previously included in an epileptic encephalopathy cohort.[9,10,33] Notably, the same nonsense variant seen in Individual 2 was identified in a (possibly) unique person in an autism spectrum disorder cohort; Individual 2 also had a diagnosis of autism.[34] One de novo missense variant was identified in a control individual; this was not located in a known functional domain and is predicted benign by PolyPhen-2 (see Fig 1).[34]

In conclusion, by gathering genetic data from multiple sites connected through GeneMatcher, we identified 24 de novo variants in *NBEA* identified

through either research or clinical genetic testing. This study implicates *NBEA* as a neurodevelopmental gene with distinctive epilepsy presentations in the first years of life, overlapping MAE phenotypes.

Acknowledgment

This publication was supported by the NIH National Center for Advancing Translational Sciences (UL1TR001873). The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH.

We thank the families that enrolled in the various research studies involved. Individual 6 was enrolled through the CAUSES study, which is funded by British Columbia Children's Hospital Foundation (Mining for Miracles) and Genome BC. Investigators in the CAUSES study include Shelin Adam, Christele Du Souich, Alison Elliott, Anna Lehman, Jill Mwenifumbo, Tanya Nelson, Clara Van Karnebeek, and Jan Friedman. We also thank individuals and groups that contributed exome sequence samples for analysis, including the Epi4K Consortium, Epilepsy Phenome Genome Project, EuroEPINOMICS-RES Consortium, Dr V. Shashi, and Dr A. Bergin.

Author Contributions

Acquisition and analysis of data: all authors. Study concept and design: T.T.S., E.L.H., D.B.G., and S.J.C.S. Drafting the text and preparing the figures: M.S.M., T.T.S., E.L.H., and N.S.

Potential Conflicts of Interest

Nothing to report.

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