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NTRK2-related developmental and epileptic encephalopathy: Report of 5 new cases

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ABSTRACT

Purpose: This study aimed to describe the phenotype of five new cases of *NTRK2*-related developmental and epileptic encephalopathy (DEE).
Methods: The clinical features, EEG, neuroimaging and genetics were reviewed for cases with likely pathogenic and pathogenic *NTRK2* variants and then summarized.
Results: Five cases of *NTRK2*-related DEE were identified. Four had a previously described recurrent variant in *NTRK2* and one had a novel variant. The phenotype was characterized by early- onset seizures (infantile spasms, later evolving to multifocal seizures), global developmental delay, variable movement disorders, microcephaly and optic nerve hypoplasia.
Conclusions: This series further expands our knowledge of the phenotype and genotype of *NTRK2*-related DEE.

Introduction

The neurotrophic tyrosine receptor kinase (*NTRK)* genes (*NTRK1, NTRK2, NTRK3*) encode a family of neurotrophic tyrosine kinase receptors, which have an important role in cell growth, cell signaling and central nervous system development.[1] Reports describing the relationship between *NTRK2* and developmental and epileptic encephalopathy (DEE) are sparse.[2] We describe the epilepsy and developmental phenotype of five newly reported children (across 5 centers in India and Canada) with pathogenic or likely pathogenic *NTRK2* gene variants. The American College of Medical Genetics (ACMG) criteria were used to classify the variants in *NTRK2*. [3,4] The clinical and genetic features of the reported cases are summarized in tables 1 and 2.

Case 1

The index case is a 7-year-old boy, born to a non-consanguineous couple. At 1 month of age, he was noted to have infantile spasms (IS) with hypsarrhythmia on EEG. He had partial response to vigabatrin but had complete electro-clinical response to ACTH (Adrenocorticotrophic hormone). At 7 months, the mother reported paroxysmal eye movements/flutter. Repeat EEG showed left frontotemporal epileptiform discharges. After 10 months, he started developing multiple daily episodes of epileptic spasms, brief tonic seizures, head drops and brief focal seizures (eye deviation to one side). These seizures were drug-resistant. He was tried on multiple anti-seizure medications (ASMs) including cannabidiol (CBD) oil. At 5 years of age, the classic ketogenic diet was started. He showed >50% improvement in his daily seizure frequency, though he continues to have daily daytime clusters of epileptic spasms and nocturnal tonic seizures. He has global developmental delay (GDD); cannot sit unassisted and is non-verbal. He also exhibited intermittent buccal dyskinesias and hemi-ballismus/choreoathetoid movements.

His other co-morbidities included visual impairment, hypophosphatemic rickets, and gastroesophageal reflux. Examination showed normal head circumference, no facial dysmorphism or

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neurocutaneous features, and central hypotonia.

His MRI brain at 2 years of age showed mild diffuse cerebral atrophy, thinning of the corpus callosum and hypoplasia of the bilateral optic nerves/optic chiasm. Serial EEGs showed multiple independent spike foci (MISF) and captured multiple spasms and tonic seizures with generalized ictal EEG findings.

Case 2

This is a 3-year-old boy born to non-consanguineous parents with no significant family or perinatal history. He presented at 6 months of age with IS and hypsarrhythmia with incomplete response to prednisolone and vigabatrin. Prior to the onset of spasms, he had head control, rolled over, had a social smile and reached for objects. There was regression of all milestones with the onset of IS. He evolved into other seizure types (focal tonic seizures, single spasms, head drops followed by right upper limb posturing). EEG also evolved into MISF. He failed multiple ASMs and continues to be drug-resistant. He also has autistic features and visual impairment (with optic disc pallor). MRI brain was unremarkable.

Case 3

This is a 3.5-year-old girl born to non-consanguineous parents with no significant family or perinatal history. She presented at 6 months of age with IS, single-spasm variant. Her seizures were subsequently controlled with valproate, clonazepam and pyridoxine. She also experienced episodic abnormal eye movements (oculogyric crisis) and choreoathetoid movements. She showed significant GDD with no head control, visual impairment and was non-verbal. Examination showed microcephaly, optic disc pallor, bipyramidal signs, choreoathetoid movements, and dystonia. MRI brain showed diffuse cerebral atrophy. Serial EEGs showed MISF.

Case 4

This is a 2.5-year-old girl born to non-consanguineous parents with no significant family or perinatal history. She presented at 7 months of age with IS and later also had myoclonic and generalized tonic-clonic seizures. She failed multiple ASMs (levetiracetam, clonazepam, oral steroids, vigabatrin, zonisamide, valproate). She also exhibited delayed milestones (can sit with support and babble at 2.5years) and autistic features. Microcephaly, strabismus and central hypotonia were noted on examination. MRI brain was unremarkable. EEG showed hypsarrhythmia and then MISF.

Case 5

This is a 11-year-old girl born to consanguineous parents (paternal grandparents were siblings). She presented at 5 months of age with microcephaly, delayed milestones, irritability and unprovoked episodic tonic posturing of limbs. A spot EEG was normal and MRI brain showed white matter paucity and hypomyelination. She continued to have episodic limb posturing, sometimes with perioral bluish discoloration; unsuccessfully treated with valproate and *ayurvedic* medications (irregular follow up). Currently, she has GDD (can sit unsupported, but is non-verbal). Examination showed microcephaly, subtle facial dysmorphism (thick lips, abnormal ears), bilateral esotropia, choreoathetoid movements, central hypotonia and ankle contractures. EEG showed background slowing with left central epileptiform abnormalities.

Extensive metabolic testing, karyotype and microarray were unremarkable for cases 1 to 5. Whole exome sequencing showed pathogenic (cases 1–4) or likely pathogenic (case 5) *NTRK2* variants. These variants arose de novo in case 1 and 5; parental testing was not performed in the other cases. The variant in case 5 (p.Arg551Gln) was classified as "likely pathogenic" based on ACMG criteria, which included the following: arose de novo, located in a critical functional domain, absent from controls in gnomAD, low rate of benign missense variation in *NTRK2* and missense variant being a common mechanism of disease, arginine being highly conserved amongst mammals and at least two protein algorithm software predicted it to be damaging (See Table 2).

Discussion

We report five novel cases of pathogenic or likely pathogenic variants in the *NTRK2* gene with a DEE phenotype via WES. Four cases (cases 1 to 4) had a previously reported recurrent variant[2] and one case (case 5) had a novel *NTRK2* variant.

The *NTRK2* gene, present on chromosome 9q21.33, encodes the TrkB protein, which is mainly expressed in the central nervous system. The

Table 1
Summary of salient features of the described cases of NTRK2-related developmental and epileptic encephalopathies.

	Current age (yrs) and sex	*NTRK2* Variant	Age at seizure onset	Seizure types	EEG	Pharmaco-responsiveness	Movement Disorder	Neurological co-morbidities	MRI
1	7/M	c.1301A>G (p. TyrY434Cys) [recurrent]	1 mo	epileptic spasms, tonic, head drops, focal	Hyps, MISF	DRE	buccal dyskinesias, hemi-ballismus, choreoathetoid movements	Central hypotonia, visual impairment	Cortical atrophy, corpus callosum thinning
2	3/M	c.1301A>G (p. TyrY434Cys) [recurrent]	6 mo	epileptic spasms, focal tonic, head drops	Hyps, MISF	DRE	NR	Visual impairment, autistic features	Unremarkable
3	3.5/F	c.1301A>G (p. TyrY434Cys) [recurrent])	6 mo	infantile spasms -single-spasm variant	MISF	Seizure free after 3 ASMs	oculogyric crisis, choreoathetoid movements, dystonia	Microcephaly, bipyramidal signs, visual impairment	Cortical atrophy
4	2.5/F	c.1301A>G (p. TyrY434Cys) [recurrent]	7 mo	epileptic spasms, myoclonic, GTCS	Hyps, MISF	DRE	NR	Microcephaly, Central hypotonia, strabismus, autistic features	Unremarkable
5	11/F	c.1652G>A (p. Arg551Gln) [novel]	5 mo	Tonic, myoclonic	Left central epileptiform abnormalities	DRE	choreoathetoid movements	Microcephaly, subtle facial dysmorphism, bilateral esotropia, central hypotonia, ankle contractures	White matter paucity, hypomyelination

DRE-drug-resistant epilepsy; F-female; GTCS-generalized tonic-clonic seizures; hyps-hypsarrhythmia; M-male; MISF-multiple independent spike foci; mo-months; NR-not reported

Table 2
NTRK2 variants seen in the reported cases.

Variant	Variant (origin)	Chromosome / coding seq / protein position	Conservation	insilico tools (SIFT, PolyPhen2)	Frequency (gnomAD)	ClinVar	HGMD	Functional domain	Variant conclusion	Supporting articles
1 (Cases 1-4)	Missense (de novo in case 1; parental testing not done in others)	GRCh37 chr9:87366905A>G/ c.1301A>G/ p.Y434C	highly conserved	Disease causing, deleterious, Damaging	Not Found	variation id: 268204	Reported (CM1716318)	beginning of the transmembrane domain	Pathogenic*	Hamdan et al 2017[4]
2 (Case 5)	Missense (de novo)	GRCh37 chr9:87549095G>A/ c.1652G>A/p. R551Q	highly conserved	Disease causing, deleterious, damaging	Not Found	not reported	Not reported	protein kinase domain; ATP binding site	Likely Pathogenic**	Not previously reported

*NTRK2 c.1301A>G: The missense variant NM_006180.6(NTRK2):c.1301A>G (p.Tyr434Cys) causes the same amino acid change as a previously established pathogenic variant [Accession ID: VCV000268204]. The p.Tyr434Cys variant is novel (not in any individuals) in gnomAD. The p.Tyr434Cys variant is novel (not in any individuals) in 1kG. There is a large physicochemical difference between tyrosine and cysteine, which is likely to impact secondary protein structure as these residues differ in polarity, charge, size and/or other properties. The gene NTRK2 has a low rate of benign missense variation as indicated by a high missense variants Z-Score of 3.73. The gene NTRK2 contains 2 pathogenic missense variants, indicating that missense variants are a common mechanism of disease in this gene. The p.Tyr434Cys missense variant is predicted to be damaging by both SIFT and PolyPhen2. The tyrosine residue at codon 434 of NTRK2 is conserved in all mammalian species. The nucleotide c.1301 in NTRK2 is predicted conserved by GERP++ and PhyloP across 100 vertebrates. The patient's phenotype matches with that of the disorder caused by pathogenic variants in NTRK2 gene. Collating all the evidence, this variant has been classified as Pathogenic.

**NTRK2 c.1652G>A: The missense variant NM_006180.6(NTRK2):c.1652G>A (p.Arg551Gln) has not been reported previously as a pathogenic variant nor as a benign variant, to our knowledge. The p.Arg551Gln variant is novel (not in any individuals) in gnomAD. The p.Arg551Gln variant is novel (not in any individuals) in 1kG. There is a small physicochemical difference between arginine and glutamine, which is not likely to impact secondary protein structure as these residues share similar properties. The gene NTRK2 has a low rate of benign missense variation as indicated by a high missense variants Z-Score of 3.73. The gene NTRK2 contains 2 pathogenic missense variants, indicating that missense variants are a common mechanism of disease in this gene. The p.Arg551Gln missense variant is predicted to be damaging by both SIFT and PolyPhen2. The arginine residue at codon 551 of NTRK2 is conserved in all mammalian species. The nucleotide c.1652 in NTRK2 is predicted conserved by GERP++ and PhyloP across 100 vertebrates. . Collating all the evidence, this variant has been classified as Likely Pathogenic.

protein has an extracellular ligand-binding domain, a transmembrane domain and an intracellular tyrosine kinase domain. It preferentially binds Brain-derived neurotrophic factor (BDNF) and Neurotrophin-4 (NT4). It acts through key downstream intracellular pathways like the Ras-ERK, PIK3, and phospholipase Cγ1 (PLC γ1) pathways, which are critical for neuron survival, proliferation, migration, differentiation, and synapse formation and plasticity.

NTRK2 activation, possibly, can have distinct biological consequences, depending on the involvement of downstream pathways. On one hand, NTRK2 confers neuroprotection, possibly through Shc-Akt downstream signaling pathway. [5] In contrast, BDNF and *NTRK2*-encoded TrkB are thought to promote epileptogenesis, mediated by the PLC γ1 downstream pathway. Consequently, TrkB kinase inhibition may exert antiseizure effects. [6] *NTRK2* gene polymorphisms have also been shown to influence age at seizure onset and pharmaco-responsiveness in patients with temporal lobe epilepsy. [7] Further, *NTRK2* was reported to be a susceptibility gene for DRE in another study. [8]

Hamdan et al [2] reported five cases of *NTRK2*-related DEEs. Four de novo cases with the p.TyrY434Cys (c.1301A>G) variant (in the transmembrane domain) were reported; a gain-of-function or dominant negative mechanism was proposed. The phenotype comprised of GDD, drug-resistant seizures (3/4 had IS), microcephaly, optic nerve hypoplasia and hypotonia. Subtle choreo-athetosis was described in one patient. This phenotype is similar to our cases (1-4). We also noted other movement disorders including buccal dyskinesias (1), ballismus (1), dystonia (1) and oculogyric crisis (1). Observation of movement disorder is not surprising as BDNF and NT-4 (preferred ligands for TrkB) are known to play critical role in modulating cortico-striatal synaptic transmission. [9] *NTRK2* adds to a long list of existing genes associated with DEE and movement disorders. [10]

The observed epilepsy phenotype in our cases was similar. Most of them had early onset seizures; infantile spasms seen in four cases. Later other seizures types were noted (tonic, spasms, myoclonic, GTCS, focal). Most (4/5) of our cases had drug-resistant seizures. EEG commonly showed MISF.

Visual impairment/optic disc pallor was also a common feature. It was seen in three of our cases and 4/5 cases in previously reported series.[2] This may be explained by possible neuroprotective role of BDNF/TrkB pathways for retinal ganglionic cells. [11] However, this is in contrast to the pure "gain-of-function" mechanism proposed for p. TyrY434Cys variant. It is unclear how a given variant effects TrkB mediated "epileptogenesis-promoting" or "neuroprotection-promoting" downstream pathways.

Case 5 had a novel variant [p. Arg551Gln (c.1652G>A)] in the intracellular tyrosine kinase domain of NTRK2 protein. The functional consequence of this variant is unknown. It may impair downstream signaling as reported with another variant (p.Tyr722Cys) in the same region. [12] Her phenotype was similar to our other cases with regard to significant developmental delay, microcephaly, drug-resistant epilepsy and movement disorders. However, EEG was more focal at the last follow up. Early onset obesity and hyperphagia described with previous variants in this region [2,12], were not noted.

Activating Trk fusions have been described in a variety of solid tumors and CNS malignancies. Gain-of-function point-mutations have also been described with hematological malignancies. None of our patients had any malignancies currently but may require surveillance for the same. Despite the role of *NTRK2* in neuronal proliferation and migration, we did not observe any cortical malformations in our cohort.

Conclusion

NTRK2-associated DEE was characterized by early onset seizures (frequently IS), GDD, variable movement disorders, microcephaly and visual impairment in our cohort. EEG frequently showed hypsarrhythmia initially and MISF later. Multi-center collaborations are warranted to describe the complete phenotype of ever-increasing list of new

genetic DEEs, including *NTKR2*.

Ethics

Informed consent was obtained from the parents of the reported cases, as per local hospital guidelines

Declaration of Competing Interest

None of the authors has any conflict of interest to declare.

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None.

References

[1] Chao MV. Neurotrophins and their receptors: a convergence point for many signalling pathways. Nat Rev Neurosci 2003;4(4):299–309.
[2] Hamdan FF, Myers CT, Cossette P, et al. High rate of recurrent De Novo mutations in developmental and epileptic encephalopathies. Am J Hum Genet 2017;101(5):664–85.
[3] Nykamp K, Anderson M, Powers M, et al. Sherloc: a comprehensive refinement of the ACMG-AMP variant classification criteria. Genet Med 2017;19(10):1105–17.
[4] Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of medical genetics and genomics and the association for molecular pathology. Genet Med 2015;17(5):405–24.
[5] Huang YZ, He XP, Krishnamurthy K, McNamara JO. TrkB-Shc signaling protects against hippocampal injury following status epilepticus. J Neurosci 2019;39(23):4624–30.
[6] Liu G, Kotloski RJ, McNamara JO. Antiseizure effects of TrkB kinase inhibition. Epilepsia 2014;55(8):1264–73.
[7] Torres CM, Siebert M, Bock H, et al. NTRK2 (TrkB gene) variants and temporal lobe epilepsy: a genetic association study. Epilepsy Res 2017;137:1–8.
[8] Almoguera B, McGinnis E, Abrams D, et al. Drug-resistant epilepsy classified by a phenotyping algorithm associates with NTRK2. Acta Neurol Scand 2019;140(3):169–76.
[9] Torres-Cruz FM, Mendoza E, Vivar-Cortés IC, García-Sierra F, Hernández-Echeagaray E. Do BDNF and NT-4/5 exert synergistic or occlusive effects on corticostriatal transmission in a male mouse model of Huntington's disease? J Neurosci Res 2019;97(12):1665–77.
[10] Spagnoli C, Fusco C, Percesepe A, Leuzzi V, Pisani F. Genetic neonatal-onset epilepsies and developmental/epileptic encephalopathies with movement disorders: a systematic review. Int J Mol Sci 2021;22(8).
[11] Chitranshi N, Dheer Y, Mirzaei M, et al. Loss of Shp2 Rescues BDNF/TrkB signaling and contributes to improved retinal ganglion cell neuroprotection. Mol Ther 2019;27(2):424–41.
[12] Yeo GS, Connie Hung CC, Rochford J, et al. A de novo mutation affecting human TrkB associated with severe obesity and developmental delay. Nat Neurosci 2004;7(11):1187–9.