Childhood-Onset Generalized Epilepsy in Bainbridge-Ropers Syndrome

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Abbreviations: DDD = Deciphering Developmental Disorders, gnomAD = Genome Aggregation Database, OIRDA = Occipital Intermittent Rhythmic Delta Activity

Summary

Bainbridge-Ropers syndrome is a genetic syndrome caused by heterozygous loss-of-function pathogenic variants in *ASXL3*, which encodes a protein involved in transcriptional regulation. Affected individuals have multiple abnormalities including developmental impairment, hypotonia and characteristic facial features. Seizures are reported in approximately a third of cases; however, the epileptology has not been thoroughly studied. We identified three patients with pathogenic *ASXL3* variants and seizures at Austin Health and in the DECIPHER database. These three patients had novel *de novo ASXL3* pathogenic variants, two with truncation variants and one with a splice site variant. All three had childhood-onset generalized epilepsy with generalized tonic-clonic seizures, with one also having atypical absence seizures. We also reviewed available clinical data on five published patients with Bainbridge-Ropers syndrome and seizures. Of the five previously published patients, three also had generalized tonic-clonic seizures, one of whom also had possible absence seizures; a fourth patient had absence seizures and possible focal seizures. EEG typically showed features consistent with generalized epilepsy including generalized spike-wave, photoparoxysmal response, and occipital intermittent rhythmic epileptiform activity. Bainbridge-Ropers syndrome is associated with childhood-onset generalized epilepsy with generalized tonic-clonic seizures and/or atypical absence seizures.

Key Words: ASXL3, Bainbridge-Ropers syndrome, Generalized epilepsy, Atypical absence, Photoparoxysmal response

1. Introduction

Bainbridge-Ropers syndrome (OMIM 615485) is caused by *de novo* heterozygous truncating pathogenic variants in *ASXL3* (OMIM 615115) (Bainbridge et al., 2013), a gene encoding a sex comb-like protein that plays a role in transcriptional regulation (Katoh and Katoh, 2004; Srivastava et al., 2016). The clinical phenotype most commonly involves severe intellectual disability, speech difficulties, autistic features, hypotonia, dysmorphic features and early feeding difficulties. Of 28 published patients, seizures were reported in 9 (Bainbridge et al., 2013; Balasubramanian et al., 2017; Contreras-Capetillo et al., 2017; Dinwiddie et al., 2013; Hori et al., 2016; Kuechler et al., 2017; Srivastava et al., 2016). Although seizures occur in ~1/3 of Bainbridge-Ropers cases, the epileptology has not yet been thoroughly characterized.

Here, we investigated the epilepsy phenotype in Bainbridge-Ropers syndrome. We report 3 patients with novel *ASXL3* pathogenic variants and seizures, as well as additional epileptology data on 5 previously published individuals.

2. Methods

We were referred a patient with Bainbridge-Ropers syndrome, and performed electroclinical epilepsy phenotyping. This study was approved by the Human Research Ethics Committee of Austin Health, Project No. H2007/02961. Informed, written consent was obtained from the subject's parents.

We reviewed clinical and molecular genetic data available on patients reported in the DECIPHER database (https://decipher.sanger.ac.uk) with *ASXL3* pathogenic variants and

seizures (Firth et al., 2009), and obtained additional clinical data from submitting clinicians. We reviewed the literature regarding seizures in Bainbridge-Ropers syndrome, and obtained additional clinical data from patients with seizures reported in a 12-patient cohort (Balasubramanian et al., 2017).

This study makes use of data generated by the DECIPHER community. A full list of centres who contributed to the generation of the data is available from http://decipher.sanger.ac.uk and via email from decipher@sanger.ac.uk. Funding for the DECIPHER project was provided by the Wellcome Trust.

3. Results

3.1 ***Patient #1***: A 6-year-old boy was referred with uncontrolled epilepsy and Bainbridge-Ropers syndrome with novel NM_030632.1:c.3106C>T, p.Arg1036* *ASXL3* variant identified on singleton whole exome sequencing. The variant was considered pathogenic, predicted to result in premature protein truncation, and not present in the Genome Aggregation Database (gnomAD) or other population databases (Lek et al., 2016). Sanger sequencing of this variant in both parents confirmed the variant was *de novo.*

His first seizure was afebrile generalized tonic-clonic status epilepticus at age two years. Valproate was started and he was seizure-free for 18 months, at which time medication was weaned. He had a brief, self-limited generalized tonic-clonic seizure at 4.5 years, but medication was not re-started.

At 5.5 years, he began to have paroxysmal episodes of impaired awareness and loss of tone (Video). With these events, his parents first noticed decreased awareness, sometimes with dysconjugate gaze, and he appeared to hold his breath. He then had slow loss of tone, fell to the floor, and injured himself with more severe episodes. The duration of events was < 30 seconds, and he had up to 40 events in a day. The frequency of events did not noticeably change with reintroduction of valproate, or initiation of clobazam and lamotrigine.

His awake interictal EEG showed normal background with no epileptiform discharges, though occipital intermittent rhythmic delta activity (OIRDA) was often seen (Figure A). The ictal recording during his impaired awareness events initially showed bilateral posterior rhythmic delta activity, which often evolved into 5-10 seconds of rhythmic bifrontal sharp-slow discharges (Figure B). Based on the clinical manifestations and EEG correlate, the events were classified as atypical absence seizures. Ethosuximide was started and the frequency of events decreased considerably.

He had generalized hypotonia and severe developmental impairment from the first year of life. He crawled at 18 months, walked independently at 4 years, and at 6 years, had no spoken words. His only episode of regression came at 5.5 years with his onset of frequent seizures. There were no autistic features. He had hypotonic facies with peri-orbital fullness, full cheeks, an open mouth with drooling and a thick lower vermillion, and small ears.

3.2 DECIPHER Patients

A DECIPHER database search found 47 patients with *ASXL3* variants identified in the Deciphering Developmental Disorders Study (DDD), although this includes patients with variants of unknown significance. Of these individuals, 5 patients with seizures have been reported previously and 2 further patients were identified with *ASXL3* pathogenic variants and seizures. The available clinical information regarding epileptology, as well as molecular genetic data, are in Table (patients #2, 3). Both patients had generalized tonic-clonic seizures alone and phenotypes consistent with childhood-onset generalized epilepsy.

3.2.1 ***Patient #2***: A 17-year-old male was evaluated at 3 months, for poor feeding, visual inattention, failure to thrive and possible seizures. Subsequently, he developed spasticity affecting his lower limbs, nystagmus, scoliosis, and multiple contractures of upper and lower limbs. He required surgical decompression for an ulnar nerve compression. His height, weight and head circumference were < 0.4th centile and he had severe global developmental impairment and was non-verbal.

In early childhood, he was investigated for stimulus-sensitive shuddering episodes with eyelid flickering, usually associated with excitement or with auditory or visual stimulation. An EEG showed generalised spike-wave discharges with photosensitivity. However, a trial of valproate resulted in overall deterioration in his behaviour, did not modify the shaking episodes, and was discontinued. Subsequent EEG telemetry captured the episodes of shaking, and suggested the events were unlikely to be epileptic. At 13 years, he presented with two episodes in a 24-hour period consistent with generalised tonic-clonic seizures, occurring during a wean of codeine

prescribed to manage generalised pain symptoms. He was commenced on lamotrigine and remained seizure-free until 16 years when he had a further episode during sleep that was thought to be a seizure. Subsequent concerns that the lamotrigine was affecting his mood led to a change to levetiracetam, discontinued at 18 years. He remained seizure-free at last assessment.

Whole exome sequencing identified a *de novo ASXL3* splice site variant (NM_030632.2:c.3039+1G>A), not present in gnomAD, and predicted to severely decrease splice signal based on the *in silico* tool, MaxEntScan (Yeo and Burge, 2004).

3.2.2 ***Patient #3***: A 7-year-old boy had mild global developmental impairment and autism spectrum disorder. He presented with prolonged generalized tonic-clonic seizures, including status epilepticus, at 4 years. Seizures typically occurred every 4-6 months. Valproate was initiated and reduced seizure frequency. His physical examination was notable for borderline microcephaly, with height and weight at ~90th percentile, and head circumference the 2nd.

Whole exome sequencing identified a *de novo ASXL3* loss-of-function variant (NM_030632.2:c.3313_3316delCAGA, p.Thr1106ArgfsTer36), not present in gnomAD.

3.3 Previously Published Cases

In the 12-patient cohort of Balasubramanian et al, one additional patient has experienced seizures in addition to the four noted in the original table. The available clinical data are summarized in Table (patients #4-8). Four patients had one or both of absence and generalized tonic-clonic seizures, with seizure types not known for one patient. One patient only had generalized tonic-clonic seizures occurring with fever; one patient was reported to have focal seizures but further details were not available. Interictal EEG results were available for one patient, showing a

normal background with both focal and generalized epileptiform discharges. Data on medication response was available for two patients; both responded to valproate with one confirmed to be seizure-free for the past two years. Notably, patient #5 had behavioural hyperventilation episodes, though these had not been associated with seizures.

4. Conclusions

We present three patients with Bainbridge-Ropers syndrome, all of whom have generalized epilepsy with childhood-onset generalized tonic-clonic seizures. Patient #1 also had unusual absence seizures, involving altered patterns of breathing that could be mistaken for breath-holding or hyperventilation events. Although detailed epilepsy phenotyping was not available for the patients published in the Balasubramanian cohort, the available data is also consistent with generalized epilepsy with generalized tonic-clonic and/or absence seizures. These findings indicate that the typical epilepsy phenotype in Bainbridge-Ropers syndrome is childhood-onset generalized epilepsy with absence and generalized tonic-clonic seizures.

Understanding the epileptology of Bainbridge-Ropers syndrome is of clinical importance, as *ASXL3* variation was frequently identified in a trio whole exome sequencing study of children with non-specific intellectual disability (Balasubramanian et al., 2017; Wright et al., 2015). A better knowledge of the typical epilepsy presentation and course will inform diagnosis, counseling and treatment decisions.

From a molecular genetic perspective, we present the first patient with a *de novo* splice site variant, as the cause of the patient's severe developmental impairment and epilepsy. All

previous cases of Bainbridge-Ropers syndrome have occurred with variants leading to premature protein truncation, as was the case with our patients #1 and 3 (Balasubramanian et al., 2017).

The observation of generalized epilepsy in Bainbridge-Ropers syndrome has important implications for our understanding of the underlying pathophysiology of this disorder. The disrupted protein, ASXL3, is involved in transcriptional regulation (Katoh and Katoh, 2004; Srivastava et al., 2016), and the recognition of the epilepsy phenotype may give clues as to which downstream pathways are disrupted. The genes implicated in the generalized epilepsies are primarily related to ion channels and neurotransmitter receptors, such as sodium channels and GABA receptors (Helbig, 2015), and it may be that altered transcriptional regulation with ASXL3 dysfunction leads to disruption of these proteins. Delineating the affected pathways could have treatment implications; however, quantitative transcriptional and functional studies are first required to confirm this hypothesis.

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boards of Neurology® and Epileptic Disorders; may accrue future revenue on a pending patent re: Therapeutic compound; has received speaker honoraria from Athena Diagnostics, UCB, GSK, Eisai, and Transgenomics; has received scientific advisory board honoraria from Nutricia and GSK, has received funding for travel from Athena Diagnostics, UCB, and GSK; and receives/has received research support from the NHMRC, ARC, NIH, Health Research Council of New Zealand, March of Dimes, the Weizmann Institute, CURE, US Department of Defense, and the Perpetual Charitable Trustees. None of the other authors have any relevant conflicts of interest to disclose.

Figure Legend

Figure - EEG recordings of Patient #1 at 6 years of age. (A) Interictal EEG shows symmetric occipital intermittent rhythmic delta activity (OIRDA). Excessive low amplitude beta activity is also noted over the anterior regions, reflecting medication effects. (B) Ictal EEG during an atypical absence seizure. There is initial rhythmic delta activity over the posterior head regions, which evolves into 6-7 seconds of slow spike-wave (~1.25 Hz) rhythmic discharges which are maximal over the frontal regions.

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