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ZMYND11 variants are a novel cause of centrotemporal and generalised epilepsies with neurodevelopmental disorder

Running Title: ZMYND11 variants and epilepsy

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CONLFICT OF INTEREST STATEMENT

All authors have been surveyed and none have any to declare.

DATA AVAILABILITY STATEMENT

All non-identifiable data and materials are available on request.

ABSTRACT

ZMYND11 is the critical gene in chromosome 10p15.3 microdeletion syndrome, a syndromic cause of intellectual disability. The phenotype of *ZMYND11* variants has recently been extended to autism and seizures. We expand on the epilepsy phenotype of 20 individuals with pathogenic variants in *ZMYND11*.

We obtained clinical descriptions of sixteen new and nine published individuals, plus detailed case history of two children. New individuals were identified through GeneMatcher, ClinVar and the European Network for Therapies in Rare Epilepsy (NETRE). Genetic evaluation was performed using gene panels or exome sequencing; variants were classified using American College of Medical Genetics (ACMG) criteria.

Individuals with *ZMYND11* associated epilepsy fell into three groups: (i) atypical benign partial epilepsy or idiopathic focal epilepsy (n=8); (ii) generalised epilepsies/infantile epileptic encephalopathy (n=4); (iii) unclassified (n=8). Seizure prognosis ranged from spontaneous remission to drug resistant. Neurodevelopmental deficits were invariable. Dysmorphic features were variable. Variants were distributed across the gene and mostly *de novo* with no precise genotype-phenotype correlation.

ZMYND11 is one of a small group of chromatin reader genes associated in the pathogenesis of epilepsy, and specifically ABPE. More detailed epilepsy descriptions of larger cohorts and functional studies might reveal genotype-phenotype correlation. The epileptogenic mechanism may be linked to interaction with histone H3.3.

Keywords

epigenetic • seizure • EEG • antiepileptic drug • comorbidity • autism • histone H3.3 • bromodomain.

Introduction

Gene variants associated with neurodevelopmental disorders are increasingly recognised as overlapping causes of both rare and common epilepsies. This overlap was first appreciated through structural genomic variation studies[1] and has been expanded by exome sequencing studies[2]: the advance in methods to interrogate the genome from structure to sequence has led to the discovery of a wide range of pathogenic mechanisms for epilepsy beyond ion- and ligand-gated channelopathies, to include chromatin remodelling proteins, transcription factors, synaptic and cell signalling proteins[3].

The progression from structural to sequence variation in locus-specific critical genes can be exemplified by the chromosome 10p15.3 microdeletion syndrome, first described in 2013, and characterised by developmental delay/intellectual disability, behavioural abnormalities, dysmorphism, hypotonia and seizures [4]. The role of *ZMYND11* as the critical gene in this region was subsequently confirmed[5-9] and the associated phenotype extended to include Cornelia de Lange syndrome[10]. *ZMYND11* encodes a transcriptional co-repressor that interacts selectively with histone H3.3, also a focus for epilepsy associated genes *CHD2*[11] and *SMARCA2*[12] and itself recently linked to autism and seizures[13]. The *ZMYND11* associated neurodevelopmental disorder phenotype was recently broadened to emphasise speech and language delay, with shared dysmorphic features[14]. However, the specific epilepsy associations with *ZMYND11* have not been described and would have implications for diagnosis and treatment. The aims of this study were to (i) identify new and published individuals with *ZMYND11* associated epilepsy; and (ii) describe the range of associated electroclinical and neurodevelopmental features.

Materials and Methods

We identified individuals from Gene Matcher[15], PubMed[45, 6, 8, 14], the European Network Therapy Rare Epilepsies (NETRE)[16], and ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/), and the clinical information was systematically summarised by local clinicians. All individuals were tested using currently available next generation sequencing technology, either through whole exome sequencing or gene panels for neurodevelopmental disorders[17], a few also with mitochondrial genome sequencing and deletion analysis (#2, #12, #16)[18]. Sequence variants were categorized according to the criteria of the American College of Medical Genetics and Genomics (ACMG)[19] and run through the Mutalyzer programme https://mutalyzer.nl/ to confirm their correct description. All new variants were then submitted to the ClinVar database https://www.ncbi.nlm.nih.gov/clinvar/

All individuals/families gave written informed consent for their data to be used in research and for publication. Two individuals are described in detail to illustrate the course of neurodevelopment and seizures. Last, we compared all described pathogenic variants by type and location to assess their correlations with epilepsy.

RESULTS

Demographics

A total of 47 individuals (16 previously unreported) aged 5-47 years were identified (Table 1 and 2), 20 had epilepsy.

Seizure semiology, EEG and Treatment Outcome

Eight individuals had definite (#2, #7, #9, #10, #19) or probable (#17, #6, 15) idiopathic focal epilepsy: of these, four had Atypical Benign Partial Epilepsy (ABPE) (#2, #7, #9, #10) and one had Rolandic epilepsy (RE) (#19) - two ABPE individuals are described below in detail; two additional individuals had probable idiopathic focal epilepsies #17 with well-controlled, childhood-onset, non-lesional focal seizures with loss of awareness and left parietal EEG origin, and #6 with focal seizures and frontotemporal and parieto-occipital EEG spikes, and another had focal seizures with loss of awareness plus preceding multiple febrile seizures (#15). Two individuals had generalised epilepsies: (#1) with probably juvenile absence epilepsy accompanied by 3-3.5Hz generalised spike-and-wave (GSW) pattern; (#16) with possible childhood absence epilepsy (lacking EEG). Two individuals had infantile onset epilepsy (#13) with multiple drug-resistant, generalised seizure types, accompanied by GSW and later disorganised background, suggesting an infantile epileptic encephalopathy (IEE), and (#14). The remaining eight individuals lacked sufficient description to ascribe an epilepsy type or syndrome. Treatment response in four individuals suggested AED resistance, while three individuals showed spontaneous remission or monotherapy control or seizure reduction. Treatment response in the thirteen other individuals is unknown.

Neurodevelopmental and neuropsychiatric findings in ZMYND11 epilepsy individuals

Neurodevelopmental disorders were universal in epilepsy associated individuals, comprising developmental delay, especially in the area of speech and language, later intellectual disability (mild-moderate 16/20; severe 4/20), autism spectrum disorder (9/20), attention deficit hyperactivity disorder (ADHD) and frequent challenging behaviour (aggression) (13/20). Notable is that autism spectrum disorder was present not only in the context of severe developmental delay or intellectual disability and that behavioural problems occurred

both with and without concern for autism spectrum disorder. Less frequent associations included micro- or macrocephaly, visual or hearing impairment. A broad but variable pattern of dysmorphism consistent with that already described[14] was present in only 12 individuals (Table 1) with some additional, previously unreported features including modified palmar crease, bushy eyebrows, thick digits and hypoplastic teeth (one individual each); the remaining eight individuals had no discernible facial dysmorphism. Neuroimaging findings revealed either no abnormalities or non-specific features: prenatal infarct, ventricular enlargement/cerebral atrophy, delayed myelination, Chiari I abnormality (n=2), glial scar; one individual had posterior polymicrogyria.

Detailed clinical description of individual #7.

Seizures. 10-year-old, right-handed girl of non-consanguineous European parents. Her younger brother had been diagnosed with autism and ADHD (not genetically tested). Seizure onset was at the age of 4 years, a focal seizure occurring 30 minutes after waking, with fixed staring, some motor rigidity then falling over to one side; the whole episode lasting 30 minutes. The second seizure was very similar to the first one and during recovery she was unable to speak. Subsequent seizures were also in the morning and included head drop over the breakfast table, and slumping of the upper body, accompanied by twitching around the mouth, extension of the arms, becoming unresponsive. Other focal seizures started with staring, followed by twitching around the mouth, opening and closing of the mouth, stiffening and jerking. She had a (possibly secondary) generalised tonic-clonic seizure at the age of nine. Her initial EEG showed electrical status in slow-wave sleep (ESES) but subsequent EEGs did not show persistence of ESES. Video-telemetry at the age of 8years and 3months showed frequent irregular multi-focal spikes over the right hemisphere with activation in

drowsiness and sleep over a normal background architecture (Figure 1). Her MRI didn't reveal a structural cause for epilepsy. She was initially treated with Sodium Valproate to which Levetiracetam was added, plus Ethosuximide following appearance of ESES.

< insert EEG Figure 1 here >

Development. Retrospectively, parental report suggested difficulties with behaviour (tantrums, stubbornness, obsessions, over activity) that had been present from 18 months of age. Academic reports from 5 years of age suggested she was making good academic progress, particularly in regard to her reading, but with difficulties in social communication and with restrictive repetitive behaviours emerging. Detailed language assessment at this time suggested average to above scores across areas of attention, listening, expressive and receptive language and overall communication. Concerns emerged around five years age about social use of language, lack of reciprocity, repetitive and restricted interests, lack of imaginative play and motivation. She could be quite passive and needed lots of prompting to ask for help and also to engage in activities of daily living, although she was physically and intellectually able to do these. She also had poor organisation, planning and adaptive functional skills.

Examination and assessment. There was no dysmorphism, and her weight, height and head size were in the average ranges. She was assessed by a multi-disciplinary team using the ADOS-2[20], ADI-R[21] and Conners-III[22] at age 7years and 7months, confirming a diagnosis of autism spectrum disorder and combined-type ADHD. Again at 10y:1mo she was assessed

using the WISC-V[UK] [23]: overall, her scores fell in the average range (full scale IQ of 87) with a relative weakness in her processing speed (standard score=80).

Daily living skills and behaviour were assessed using the Adaptive Behaviour Assessment System Third Edition (ABAS-III)[24]. This revealed ratings in the Extremely Low range across Conceptual, Social and Practical domains (0.2nd centile) indicating significant functional impairment consistent with a diagnosis of autism spectrum disorder.

Behaviour and mood. She became anxious and would have temper tantrums if she didn't know what was going to happen next. She experienced long periods of low mood, perhaps explained by difficulty in coping with the stress of everyday life, including anxiety around changes and transition, the effort needed to manage with tasks of daily living, and particularly the social, academic and organisational demands at school - becoming overwhelmed by her environment. Ratings on the Teacher version of the Strengths and Difficulties Questionnaire (SDQ)[25] confirmed significant impact of her neurodevelopmental diagnoses on peer relationships and classroom learning, as well as some concerns with high emotional symptoms.

In summary, her electroclinical picture was consistent with Atypical Benign Partial Epilepsy and her neurodevelopmental profile of autism and ADHD with broadly average range IQ with low adaptive behaviour skills (Table 1).

Detailed clinical description of individual #10.

A 5-year-old girl born at term after an uneventful pregnancy to nonconsanguineous, healthy parents. She was first referred for a neuropediatric evaluation at the age of 6 months due to divergent strabismus of the left eye. Her development was marked by a global hypotonia, with generalized hyperlaxity and moderate to severe psychomotor delay: sitting at the age of 9 months and walking at 26 months. Her cognitive development was normal except for limited expressive language skills. At the age of 2 years and 9 months, she started to have atonic seizures, atypical absences with eyelid myoclonia, as well as occasional myoclonia of the limbs. Throughout the course of her disease, she has had repeated clusters of seizure exacerbation, typically manifest by prolonged epochs of eyelid myoclonia with backward head drop. These were often accompanied by cognitive and language regression lasting up to several days. At last follow-up visit, at 5 years, the parents reported the appearance of a new type of seizure, with focal myoclonias involving the right arm exclusively. On examination, the right hand was used much less spontaneously than the left, but no objective paresis or tone abnormality was noted. Currently, her global development was progressing regularly, but certain difficulties persisted mainly in the area of expressive language, balance, and fine motor skills. So far unsuccessful treatment approaches include valproic acid, levetiracetam, and a classic 4:1 ketogenic diet.

< insert EEG Figure 2 here >

The first awake EEG at 3 years and 2 months showed focal and bilateral, frontal predominant spike-wave discharges at 2.5 Hz, with left hemispheric emphasis, at times (but far from always) correlated with clinical atypical absences. The background rhythm was normal. This EEG and clinical presentation is consistent with an early onset Idiopathic focal epilepsy such

as atypical benign partial epilepsy syndrome in which atonic-astatic seizures, atypical absences, and more rarely, myoclonic seizures have been described[26]. The prolonged EEG at 3 years and 9 months showed left frontal intermittent slowing in the awake state. This was also the area of the most prominent epileptogenic focus with left fronto-central spike/polyspike-and-wave discharges, which were also seen bilaterally. Also noted in wakefulness were more diffuse and bilateral 2-2.5Hz spike/polyspike-and-wave discharges; lasting 1-3 seconds in duration. These discharges were bilaterally synchronous and more widespread over the left hemisphere and were at times clinically associated with atonic seizures with eyelid myoclonus. In sleep, the frequency of the bilaterally synchronous 1-2Hz spike-and-wave discharges increased to become almost continuously present, occupying more than 50% of the trace, and thus representing Electrical Status Epilepticus during slow-wave Sleep. Generalized faster frequency spike-wave or polyspike-wave discharges were not observed. Tonic seizures were not seen. The presence of slowing in the main epileptogenic site and a tendency of the focal discharges to extend to adjacent areas, especially to the other hemisphere in wakefulness with occurrence of new seizure types can be indicators of developing ESES[27]. This EEG progression with clinical deterioration appears to be within the severe end of the spectrum of Idiopathic focal epilepsies, classified as Encephalopathy with ESES or CSWS syndrome[28]. The initial magnetic resonance imaging at 4 years was normal, repeated at 5 years because of the focal findings described above, and again normal.

< insert Table 1 and 2 here >

Genetics.

Forty-three variants were identified in the literature, not including copy number variants. Many different types of variants were identified, including missense, frameshift, deletions, protein truncations and splice site variants (Figure 3). All four ZMYND11 domains contained pathogenic or likely pathogenic variants and there was no correlation with the presence/absence of epilepsy and the type or location of variants. The PHD Zn finger domain contained frameshift variants only, but the other domains contained a mixture of variants: frameshift and protein truncation in the bromodomain, missense and protein truncation in the PWWP domain and missense, deletions and protein truncation in the MYND Zn finger domain. The vast majority of variants were *de novo*, which is perhaps expected given what we know about other rare and severe genetic epilepsies and neurodevelopmental disorders; however, there was evidence of both maternal and paternal transmission. In the case of paternal transmission, the father had mild learning difficulties himself and his variant was identified in blood. In the five cases of maternal transmission however, two mothers were found to be mosaic for the variant, three others were affected. We were not able to identify the inheritance of all of the variants. The phenotype produced by each different genotype was varied in terms of symptoms, severity, age of onset and prognosis, although common themes were obvious including developmental delay/intellectual disability, autism spectrum disorder, behavioural issues, facial dysmorphism and seizures. This provides evidence for reduced penetrance and variable expression but not for any reliable genotype-phenotype correlations.

< insert Figure 3 here >

DISCUSSION

This study refines the association of *ZMYND11* variants with specific types and syndromes of epilepsy that have not previously been documented. *ZMYND11* variants have a particular association with a rare epilepsy, atypical benign partial epilepsy, a type of idiopathic focal epilepsy with EEG centrotemporal spikes. Our findings suggest that facial dysmorphism and intellectual disability may occasionally be absent in *ZMYND11* associated neurodevelopmental disorder although other neurodevelopmental deficits are invariable. *ZMYND11* joins a small but growing group of other monogenic epilepsy genes involved in chromatin remodelling such as *CHD2* and *SMARCA2*. The mechanism of epileptogenesis in *ZMYND11* is unknown but may result from dysregulation of neuronal differentiation in specific brain areas during the embryonic period, or of neuronal connectivity, maturation or activity during early postnatal life. Similarity is noted to the phenotype of *H3F3A* and *H3F3B* variants[13], suggesting that histone H3.3 interactions play a role in pathogenesis.

Phenotypes. The *ZMYND11* associated phenotype has been described as including developmental delay, particularly affecting speech, mild-moderate intellectual disability, significant behavioural abnormalities, hypotonia and a shared pattern of dysmorphism comprising prominent eyelashes and eyebrows, depressed nasal bridge and bulbous tip, anteverted nares, thin upper vermilion and wide mouth[14]. In this study, we show a much broader and milder phenotype may exist, that developmental delay and intellectual disability are not invariable, that neurological findings can be normal, and that facial dysmorphism may be absent. Autism is also a common association, not only associated with severe developmental delay.

Particularly interesting is the association of pathogenic *ZMYND11* variants with ABPE, which closely resembles the more common RE or childhood epilepsy with centrotemporal spikes in its initial presentation of nocturnal focal seizures affecting the vocal tract, but is much rarer, has an earlier age of onset (4-7 years), also features focal motor, myoclonic, myo-atonic or atypical absence seizures and can run a more severe seizure course with deficits in language and social communication as well as transient drug resistance[29, 30]. ABPE can be mistaken by non-specialists for other rare childhood epilepsies such as Lennox-Gastaut syndrome or myoclonic-atonic epilepsy.

The known genetics of epilepsies in idiopathic focal epilepsies include structural rearrangements, coding and non-coding variation. Recurrent and novel chromosomal rearrangements at 16p11.2, 16p13.11, 1p36, Xp22.31 constitute risk factors for RE and ABPE[31, 32]. Both inherited and *de novo* variants in the NMDA receptor subunit *GRIN2A* are found in up to 20% of ABPE, LKS and ECSWS cases[33-35]. One rare case of myoclonic atonic epilepsy evolving to ABPE has been attributed to an *SLC6A1* variant[36]. GWAS demonstrates that the EEG abnormality of CentroTemporal Spikes (CTS) in RE is caused by 3'UTR variants disrupting the developmental regulation of the *PAX6* gene by miRNA328 [37]. *ZMYND11* is the first gene involved in chromatin remodelling associated with idiopathic focal epilepsies.

Genotype-phenotype correlation. *ZMYND11* is a highly conserved gene across hundreds of vertebrate and invertebrate species and is relatively intolerant to sequence variation (RVIS score: -0.49). Pathogenic variants (ACMG IV/V) are distributed across N-terminal, PHD domain, bromodomain, PWWP domain, MYND domain and C-terminal regions; there are no clear distinctions between epilepsy and non-epilepsy associated residue distributions (Figure

2). Neither is there any clear association between (frameshift/truncation/splicing) variant type and phenotype severity, most of which are predicted to result in a loss-of-function mechanism via premature truncation and/or nonsense-mediated decay[14]. Functional studies are required to explain phenotypic heterogeneity.

***ZMYND* function**. The ZMYND proteins are part of the bromodomain family[38] and contain conserved C-terminal MYND domain, PWWP domain, bromodomain and N-terminal PHD type zinc finger domain; the combination of domains confers the ability to recognise multiple types of histone modification[39]. ZMYND11 forms an oligomer through its C-terminus; the PHD and MYND domains are important for nuclear localisation and for sumoylation; the PHD domain plays an indispensable role in inhibiting neuronal differentiation[40]. *ZMYND11* is ubiquitously expressed[41] and shows no region specificity in mammalian brain[41], present across several brain areas[42] including the hippocampus, midbrain and basal ganglia, and across developmental stages[43]. *ZMYND11* up-regulated genes are specifically enriched in small cell lung cancer and focal cell adhesion pathways[44]. Yet the predominant brain phenotype of variants suggests an important but as yet undefined role in human brain development and function. *ZMYND11* expression is enriched during development in human somatosensory cortex and is thought to play a role in the development and diversity of corticothalamic projection neurons [45], which broadly control access of sensory information to the cortex by modulating the activity of the thalamus, and locally regulate the activation state of other types of neurons across cortical layers in their area[46, 47].

***ZMYND* putative disease mechanism.** *ZYMND11* is an established intellectual disability gene MRD30, OMIM 616083 [6] and a candidate autism gene[5], implicated in anxiety-like behaviour

in outbred mice [48]. Most cases are *de novo* but inherited autosomal dominant cases have also been reported, including parental mosaicism. The mechanism by which *ZMYND11* variation causes epilepsy is currently unknown but may be connected to *ZMYND11's* role in regulating neuronal differentiation[40], as for the chromatin remodeller and epilepsy gene *CHD2*[49]. The paralog *ZMYND8* gene is also a transcriptional co-repressor and histone binding protein but has selective interaction with H3.1K36me2/H4K16ac (demethylated lysine 36 on histone 3.1/acetylated lysine 16 on histone 4), this selectivity believed to be determined in part by replacement of amino acid residues N263 and R265[50]. *ZYMND8* is also associated with neurodevelopmental disorders and seizures [6 51, 52] and is widely expressed in the *Xenopus* embryonic nervous system, overexpression inhibiting neuronal marker expression and leading to severe neural tube defects, suggesting a similar role to *ZMYND11* in regulating neural differentiation[53].

Alternatively, *ZMYND11* variants might cause epilepsy by having a more direct role in regulating the postnatal maturation and function of neurons. ZMYND11 is a H3.3-specific chromatin reader of H3K36me3 (trimethylated lysine 36 on histone 3.3)[44] and is proposed not as an "on-off switch" but as a "fine-tuner" of gene expression, colocalising in the nucleus with highly expressed genes, and functioning as a co-repressor in normal and neoplastic conditions by inhibiting Pol II-mediated transcriptional elongation. Among its multiple interaction partners are c-Myb and N-CoR[54, 55]. H3.3 accumulates in neurons as they differentiate, with H3.3 incorporated into nucleosomes along the body of highly expressed genes in an activity-dependent manner. H3.3 incorporation appears to be most critical for the expression of genes associated with synaptic development, function and plasticity. Accordingly, reduced H3.3 expression is associated with reduced excitatory synapses and

reduced miniature excitatory postsynaptic currents (mEPSCs) in cortical pyramidal cells[56]. Thus, if ZMYND11 also functions as an inhibitor of transcriptional elongation in neurons, one might predict that a reduction in ZMYND11 function would lead to increased expression of these same genes as a result of increased transcriptional elongation and increased excitatory drive, which may underlie the predisposition of patients with *ZMYND11* variants to epilepsy.

Chromatin readers/remodellers and epilepsy. Variants in several classes of chromatin readers/remodellers are associated with neurodevelopmental disorders and epilepsy. *SMARCA2* is one of the genes that encodes the catalytic subunit components of the SWI/SNF complex. SWI/SNF variants are implicated in a range of neurodevelopmental disorders such as Coffin–Siris syndrome, sporadic intellectual disability, autism spectrum disorder, schizophrenia, Kleefstra syndrome and myoclonic-atonic epilepsy [12, 57, 58]. CHD proteins are also important in neurodevelopment, with pathogenic variants in *CHD1, CHD2* and *CHD8* associated with autism spectrum disorder, intellectual disability and epilepsy, *CHD4* with congenital deafness and intellectual disability and *CHD7* with CHARGE syndrome [49, 59-61]. There is overlap with the *H3F3A/H3F3B* mutation phenotype, which is variable and includes severe developmental delay, growth abnormalities, abnormalities of tone, seizures, neurodegeneration, dysmorphism and minor congenital abnormalities[13].

Chromatin remodellers play an essential role in regulating the development of the nervous system through the establishment and maintenance of neural cell identity, by promoting the transition from relatively open chromatin states conducive to transcriptional activity in stem and progenitor cells to repressive chromatin states for genes associated with alternative cell fates that occur during the execution of neural lineage-specific differentiation programs[62].

They also play key roles in controlling the balance between proliferation and differentiation. Furthermore, chromatin remodelling factors that regulate activity-dependent gene regulation in postmitotic neurons may be essential for maintaining normal brain connectivity and the balance between excitation and inhibition [63]. Further experimentation in model systems will be necessary to identify the salient mechanisms responsible for epilepsy associated with *ZMYND11* variants.

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