Fragile Females: Case Series of Epilepsy in Girls With *FMR1* Disruption

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Girls with pathogenic variants in *FMR1*, the gene responsible for Fragile X syndrome, have received relatively little attention in the literature. The reports of girls with trinucleotide expansions or deletions affecting *FMR1* describe variable phenotypes; having normal intelligence and no severe neurologic sequelae is not uncommon. We reviewed epilepsy genetics research databases for girls with *FMR1* pathogenic variants and seizures to characterize the spectrum of epilepsy phenotypes. We identified 4 patients, 3 of whom had drug-resistant focal epilepsy. Two had severe developmental and epileptic encephalopathy with late-onset epileptic spasms. Our findings demonstrate that *FMR1* loss-of-function variants can result in severe neurologic phenotypes in girls. Similar cases may be missed because clinicians may not always perform Fragile X testing in girls, particularly those with severe neurodevelopmental impairment or late-onset spasms.

Fragile X is a genetic syndrome most typically caused by trinucleotide (CGG) expansions in *FMR1*[1] and is considered the most common known inherited cause of intellectual disability and autism.[2] Classic features include a long face, large ears, macrocephaly, macroorchidism, and behavioral abnormalities. The syndromic features classically occur in boys, but girls who are heterozygous for the full mutation expansion (>200 CGG repeats) are frequently affected, having intelligence that typically ranges from normal to moderate intellectual disability.[3] Deletions affecting *FMR1* are an infrequent cause of Fragile X[4]; however, the phenotype may be more complex if the deletion is large and other genes are affected, resulting in a contiguous gene deletion syndrome.[5] Girls rarely have Fragile X syndrome due to deletions affecting *FMR1* because of both the rarity of such deletions and the fact that larger deletions are typically associated with X-inactivation that is skewed such that the deleted allele is preferentially inactivated[5,6]; in these cases, neurodevelopmental impairment is generally mild, and presentation most commonly involves infertility or premature ovarian failure.[7]

Boys with Fragile X syndrome have an increased risk of epilepsy, but still, only 14% to 18% have seizures.[8,9] Studies of girls with *FMR1* expansions suggest seizures are even less common, and when they do occur, they are mild; a parental survey involving 304 girls with *FMR1* full mutation found that only 6% had seizures, and none were taking >1 antiepileptic drug.[8] There are fewer data regarding girls with deletions affecting *FMR1*, but even the rare patients reported with more severe developmental impairment have not had epilepsy.[6,10,11]

Here, we describe 4 girls with heterozygous *FMR1* loss-of-function variants; 2 had severe developmental and epileptic encephalopathy with

abstract

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Dr Myers conceptualized and designed the study and drafted the initial manuscript; Drs van 't Hof, Sadleir, Legault, Simard-Tremblay, Amor, and Scheffer assisted with data collection; and all authors reviewed and revised the final manuscript, approved the final manuscript as submitted, and agree to be accountable for all aspects of the work.

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multiple seizure types, including epileptic spasms.

METHODS

We retrospectively reviewed 3 research databases, the Neurodevelopmental Disorders Biobank (Research Institute of the McGill University Health Centre, Montreal, Canada), the Genetic Basis of Epilepsy Database (Austin Health, Heidelberg, Australia), and the Epilepsy Research Group database (University of Otago, Wellington, Wellington, New Zealand), for cases of girls with *FMR1* pathogenic variants and seizures. The databases include a total of ~11 000 patients who have had seizures and store clinical data, including results of any genetic testing. Thorough epilepsy phenotyping was performed in all cases, and data were updated whenever new clinical information became available. All patients or their parents and/or legal guardians gave informed written consent. This study was approved by the local research ethics boards in all cases.

Patient 1

A 13-year-old girl had refractory epilepsy and moderate developmental impairment. Seizures began at 4 years of age and initially involved generalized clonic movements with impaired awareness and urinary incontinence (Table 1). When assessed with video-EEG monitoring for new clinical events at 9 years, she was found to have epileptic spasms with brief head drops and blinking, and EEG showed a bilateral spike-wave burst followed by electrodecrement.

Her awake EEG showed multifocal epileptiform discharges with normal background activity at 7 years, and by age 10 years, she had continuous spike-wave in sleep. She had partial clinical response to valproate, clobazam, rufinamide, lamotrigine, and sulthiame, but no regimen completely controlled seizures. Levetiracetam and oxcarbazepine were discontinued because of adverse events, whereas topiramate and intravenous immunoglobulin were stopped because of a lack of efficacy. The ketogenic diet elicited clinical improvement in seizure control and cognitive function.

Developmentally, she was globally delayed from birth and had moderate intellectual disability. She had several instances of regression after clusters of seizures. Her parents reported severe attentional and behavioral issues, and she was treated with aripiprazole, methylphenidate, clonidine, and escitalopram. She also had pendular horizontal nystagmus from birth. There was no known family history of seizures or developmental impairment.

Genetic testing done at 7 years of age revealed a large *FMR1* expansion with ~620 CGG repeats (normal range 5–40). Brain MRI results, also at 7 years of age, were normal.

Patient 2

A 12-year-old girl had refractory epilepsy including late-onset epileptic spasms. Her epilepsy began at 2 years with febrile generalized tonic-clonic seizures followed by occasional brief afebrile generalized tonic-clonic seizures. She was started on valproate and became seizure free by 3 years. At 9 years, frequent seizures returned. She had daily tonic seizures with impaired awareness, usually occurring when she was taking a shower. She also had several episodes of tonic or tonic-clonic status epilepticus and 1 episode of nonconvulsive status epilepticus. In addition, she had daily focal impaired awareness seizures involving staring with manual automatisms, and daily clusters of head nods, consistent with epileptic spasms. Her epilepsy was refractory, although she appeared to have partial response to valproate, lamotrigine, and levetiracetam.

Video-EEG monitoring at age 10 years showed right central interictal epileptiform discharges that attenuated with physical activity or hand movements and independent biposterior temporal epileptiform discharges in sleep. Frequent focal seizures in sleep were captured, comprising erratic movements with head deviation to the left, with EEG showing a bitemporal discharge followed by a decrement evolving to a run of discharges.

Development was delayed from the age of 4 to 6 months. She walked after 2 years, and her speech was clearly delayed at age 2 years. Regression began at 3 years of age, and development continued to decline over the following years. A psychological assessment at 9 years found moderate intellectual disability. In addition, there were behavioral problems with daily tantrums. Her medical history was otherwise unremarkable apart from mild scoliosis.

Her maternal grandfather had tonic-clonic seizures starting at age 20 years, but was suspected to have had milder seizures as a toddler, and had learning difficulties in school. The patient had 1 maternal half-brother who was healthy, although there had been concerns about his development.

Brain MRI at ages 3 and 10 years showed minor sulcal prominence. Chromosome microarray done at 4 years of age revealed a de novo ~6.1 Mb heterozygous deletion of the X chromosome (arr[hg19] Xq27.3q28 [142184543–148268501]x1), comprising 13 Online Mendelian Inheritance in Man (OMIM) genes, including *FMR1* (Fig 1).

Patient 3

A 26-year-old woman had focal impaired awareness seizures from age 10 years. Her seizures typically involved inappropriate giggling for 2 minutes followed by 30 seconds of

TABLE 1 Clinical and Genetic Data for Girls With *FMR1* Disruption

No. and Age at Study	Age of Seizure Onset, y	Seizure Type	Medication Response	EEG	Development	Genetic Abnormality (Inheritance)
1, 13 y	6	FIAS, ES	VPA, clobazam, rufinamide, lamotrigine partially effective; AE with levetiracetam and oxcarbazepine; topiramate ineffective	Multifocal EDs, CSWS	Moderate ID, globally delayed from birth	*FMR1* CGG full expansion, 620 repeats (maternal)
2, 12 y	2	FS, GTC, FIAS, ES, tonic, NCSE	VPA, lamotrigine, levetiracetam partially effective	Multifocal EDs (right central, right and left posterior temporal)	Moderate ID, global delay apparent from 4 to 6 mo, regression at 3 y	~6.1 Mb Xq27.3–Xq28 deletion (de novo)
3, 26 y	10	FIAS	Lamotrigine partially effective; VPA, levetiracetam, topiramate, clobazam ineffective; carbamazepine rash	Normal	Severe ID	~5.9 Mb Xq27.2–Xq28 deletion (de novo)
4, 4 y	4	Focal to GTC status epilepticus	Seizure-free for 12 mo on clobazam	Normal (sedated)	Mild-moderate global delay, ASD	*FMR1* CGG full expansion, 730 repeats (maternal presumed)

AE, adverse event; ASD, autism spectrum disorder; CSWS, continuous spike-wave pattern in sleep; ED, epileptiform discharge; ES, epileptic spasm; FIAS, focal impaired awareness seizure; FS, febrile seizure; GTC, generalized tonic clonic; ID, intellectual disability; NCSE, nonconvulsive status epilepticus; VPA, valproic acid.

staring with unresponsiveness and slight head turn. Postictally, she would jump up and down and appear unsteady. Seizures occurred up to several times per day and clustered around menses. She had a single focal to bilateral tonic-clonic seizure. She was initially tried on valproate with no effect, and carbamazepine produced a rash. Lamotrigine resulted in seizure freedom for a year at age 13 years. Unfortunately, frequent seizures returned and have been unresponsive to lamotrigine, levetiracetam, topiramate, and clobazam.

She first presented with developmental concerns at 18 months because she was not walking. She had no regression but continued to have slow global developmental progress. She walked at 20 months and had her first words at 2.5 years. She has severe intellectual disability.

She had multiple EEGs, all of which had normal results. Brain MRI results were normal on 2 occasions. Chromosome microarray done at 19 years of age revealed a de novo ~5.9 Mb deletion of the X chromosome (arr[hg19] Xq27.2q28 [141342242–147272946]x1), comprising 13 OMIM genes, including *FMR1* (Fig 1).

Patient 4

This 5-year-old girl presented with generalized tonic-clonic status epilepticus lasting ~90 minutes at 4 years of age. The event occurred at day care, and workers reported abnormal breathing and movements before generalized convulsions, so focal onset was suspected. She was treated with rectal diazepam, intravenous lorazepam, and

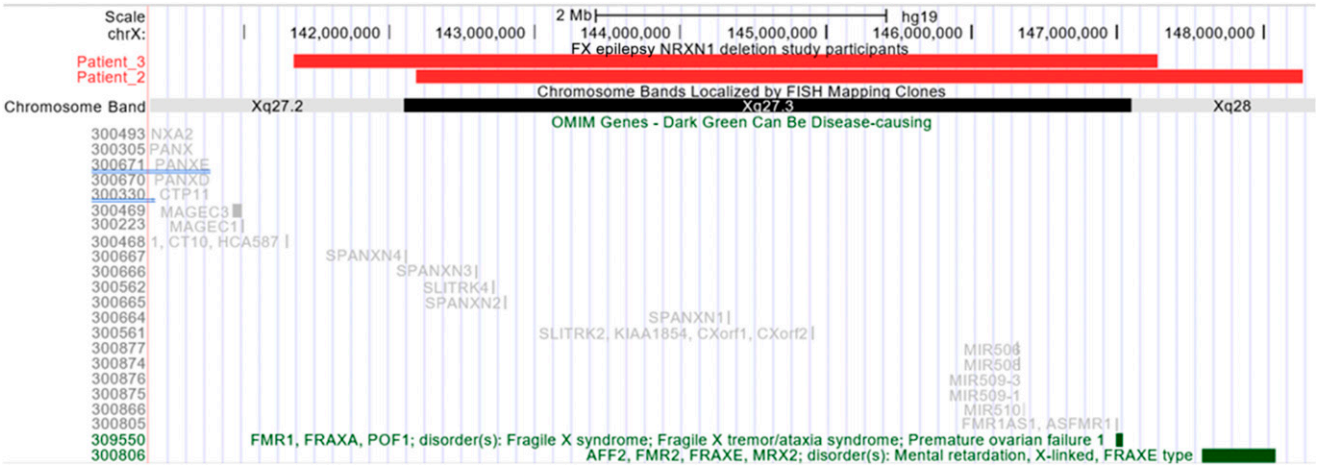

FIGURE 1
Deletion details for patients 2 and 3, including a screenshot from UCSC genome browser (http://genome.ucsc.edu), (hg 38). chrX, chromosome X; FISH, fluorescence in situ hybridization; FX, fragile X.

intravenous diazepam before the seizure stopped. She required intubation and admission to the ICU. There was no intercurrent illness or other apparent provoking factor. She was prescribed clobazam and has had no additional seizures in the 12 months since her presentation.

Her development has been delayed from 18 months of age, with deficits noted primarily in language and social function, and she was diagnosed with autism spectrum disorder. She walked around 18 months and has difficulty with fine motor tasks.

There is no known family history of seizures. A maternal male cousin has Fragile X syndrome.

She had normal head computed tomography results after the status epilepticus and brain MRI showed only a pineal cyst not thought to be clinically important. An EEG performed under chloral hydrate sedation after the status showed normal sleep architecture without epileptiform abnormalities. The chromosome microarray result was normal, but Fragile X testing at 4 years of age identified a heterozygous *FMR1* expansion of ~730 CGG repeats.

DISCUSSION

In this study of 4 females with deletions or full mutation expansions of *FMR1*, all had focal epilepsy with 3 being drug resistant. Two of these 3 had severe DEE involving multiple seizure types including late-onset epileptic spasms diagnosed at 9 years of age. Two had full mutation expansions of the *FMR1* CGG allele, whereas 2 had large deletions of the Xq27.3–Xq28 region, with epileptic spasms occurring in 1 girl with each type of pathogenic variant. These findings are surprising given that existing literature suggests that females with heterozygous *FMR1* loss-of-function variants more often have milder neurodevelopmental phenotypes. In our patients, it remains unclear why their epilepsy phenotypes were much more severe than even what has been described in males with Fragile X syndrome.

One possible explanation for the severe epilepsy in these girls may lie in the downstream gene regulation of the Fragile X protein. FMRP is a selective RNA-binding protein that associates with polyribosomes and is likely involved in translation regulation.[12] A large-scale epileptic encephalopathy genetic study found that de novo pathogenic variants tend to be most over-represented in the set of genes regulated by the Fragile X protein.[13] Further study, possibly involving transcriptional analysis, is necessary to better delineate the molecular pathways disrupted in those individuals who develop severe epilepsy phenotypes.

Epilepsy in males with Fragile X is most commonly focal, with centrotemporal spikes being the most common EEG finding.[9,14] All 4 of these females had focal seizures as well, and 1 had continuous spike wave in sleep on EEG. Taken together, these findings suggest that some epilepsy phenotypes in patients with *FMR1* loss-of-function variants fall on the epilepsy-aphasia spectrum.[15]

A likely contributing factor for the lack of published reports of females with *FMR1* loss-of-function variants and severe neurodevelopmental phenotypes is that diagnoses are missed because affected individuals never receive *FMR1* CGG expansion testing. Loss-of-function *FMR1* variants have not previously been reported with DEE in females, so clinicians often omit *FMR1* testing from their diagnostic workup in female patients. This is even more likely in the current genetic testing environment, in which clinicians often order only chromosome microarray and/or next-generation sequencing gene panels, without considering *FMR1* expansions in females. Our findings emphasize that CGH microarray and *FMR1* expansion testing should be performed in all patients with developmental impairment and epilepsy, male or female.

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ABBREVIATIONS

DEE: developmental and epileptic encephalopathy
OMIM: Online Mendelian Inheritance in Man

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