ELSEVIER

Seminars in
Pediatric
Neurology

Fraternal Twins With Autism, Severe Cognitive Deficit, and Epilepsy: Diagnostic Role of Chromosomal Microarray Analysis

Jaime Imitola, MD,[*] Diana Walleigh, MD,[†] Carol E. Anderson, MD,[‡] Reena Jethva, MD, MBA,[†,‡] Karen S. Carvalho, MD,[†] Agustin Legido, MD, PhD, MBA,[†] and Divya S. Khurana, MD[†]

A 7-year-old child presented with atypical absence epilepsy. He also had autism and severe cognitive deficit. As part of his diagnostic workup, a chromosomal microarray analysis was performed, which showed novel biallelic deletions in the neurexin 1 gene (*NRXN1*). His fraternal twin sister, who also had autism and cognitive impairment, was subsequently found to have the same biallelic deletions. Deletions included a 272-282 kb loss at band 2p16.3 in one allele and a smaller 135-174-kb loss on the second allele. Neurexin 1 (NRXN1) is a cell adhesion protein, forming a synaptic complex with neuroligin. This signals a pathway that is critical for activity-dependent synaptic transmission. Mutations in this gene have been associated with autism and neurodevelopmental delay. Although there are many reports of heterozygous mutations with variable expressivity, only 3 cases with biallelic *NRXN1* mutations have been previously reported, all of which have a more severe phenotype. We report 2 siblings with biallelic deletions, both of which affect the promoter region and exons 1-5 in the α-*NRXN1* isoform, which has a role in the Ca^{2+}-dependent release of neurotransmitters in the central nervous system. Our cases expand the phenotype of biallelic *NRXN 1* mutations and emphasize the important role of NRXN1 in autism and intellectual disability. Chromosomal microarray analysis should be the clinical standard in all specialties for first-tier genetic testing in autistic spectrum disorders.
Semin Pediatr Neurol 21:167-171

Case 1

The propositus was a full-term male child of an uneventful twin gestation pregnancy. Parents were healthy, nonconsanguineous white individuals, with no family history of developmental delay or cognitive deficits, seizures, autism, or mental health problems. He had a history of ventricular septal defect. Psychomotor development was severely delayed for motor skills (he sat up at 1 year and walked at 2 years) and for language. He also developed clinical features compatible with the diagnosis of autistic disorder. At the age of 7 years, he started to present with episodes of staring and stiffening at school. Electroencephalogram monitoring showed atypical absence seizures consistent with generalized epilepsy (Fig. 1). His language consisted of approximately 2-10 words. He had outbursts of aggressiveness and self-injurious behavior that involved punching himself on the neck and choking himself. Other symptoms included dysphagia, constipation, stereotypies, and abnormal sleep-wake cycle. His height was at the fifth percentile, and weight was at the tenth percentile. Head circumference was between the 25th and the 50th percentiles. Brain magnetic resonance imaging scan revealed no structural brain abnormalities. The seizures were treated with administration of 50 mg of topiramate and 250 mg of sodium valproate, twice a day each, and the frequency of seizures decreased with the antiepileptic therapy. His behavioral problems were treated with risperidone and

From the Division of Neuroimmunology and Multiple Sclerosis, Department of Neurology and Neuroscience, Wexner Medical Center, The Ohio State University, Columbus, OH.
†Section of Neurology, Department of Pediatrics, St. Christopher's Hospital for Children, Drexel University College of Medicine, Philadelphia, PA.
‡Section of Genetics, Department of Pediatrics, St. Christopher's Hospital for Children, Drexel University College of Medicine, Philadelphia, PA.
Address reprint requests to Divya S. Khurana, MD, Section of Neurology, Department of Pediatrics, St. Christopher's Hospital for Children, 3601 A St, Philadelphia, PA 19134. E-mail: divya.khurana@drexelmed.edu

1071-9091/14/$-see front matter
http://dx.doi.org/10.1016/j.spen.2014.04.027

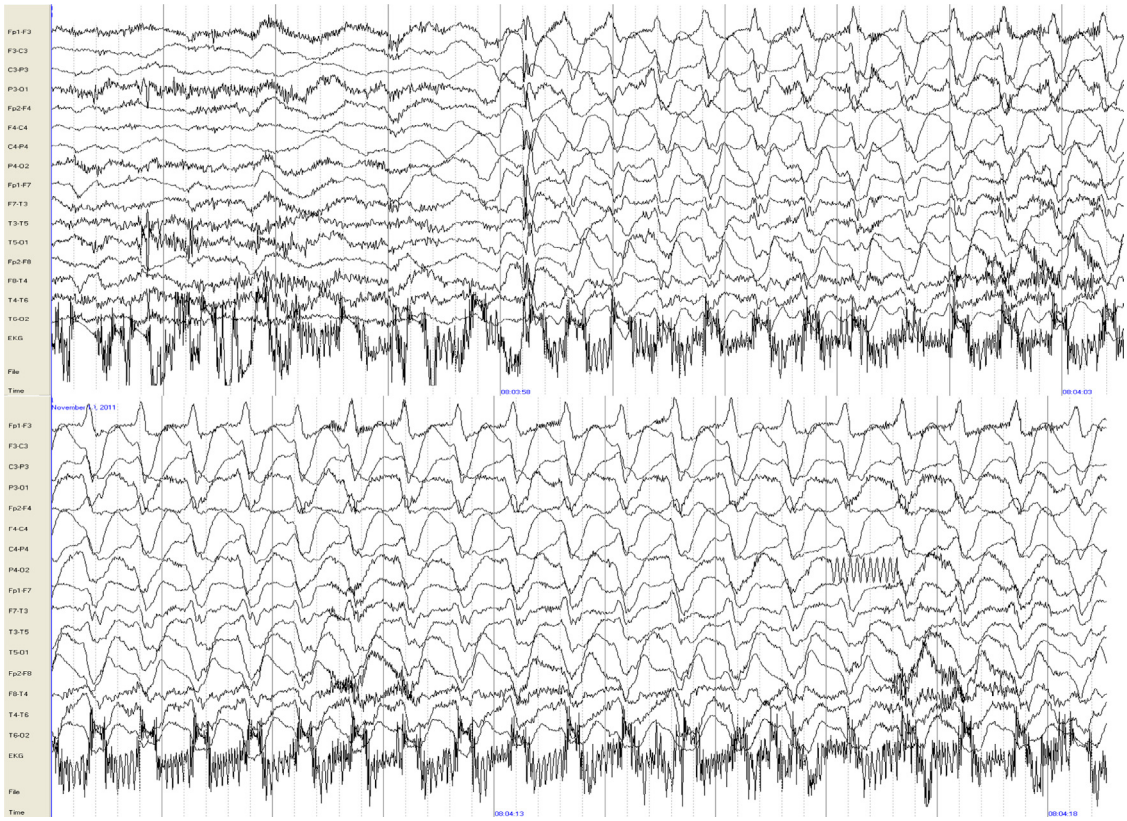

Figure 1 A representative EEG of patient 1 showed generalized paroxysmal spike wave at the onset of a staring spell followed by high-amplitude (up to 200 μV) rhythmic 2-Hz notched delta activity with duration of 40 seconds to 1 minute. Additional EEGs showed slow background for age. EEG, electroencephalogram.

extended-release guanfacine, in addition to behavioral therapy, with little benefit.

Case 2

The fraternal twin sister of the propositus was also born at full term with no medical issues. However at 6 months of age, she showed an inability to play and interact with others. She was later diagnosed with severe developmental delay and language impairment. Her clinical progress was very slow. At 7 years, she had some vocalizations, and her language was limited to 2-5 words. She had strabismus, hypotonia, and ataxia. Other symptoms included hyperventilation, stereotypies, constipation, and abnormal sleep-wake cycle. Her height was at the fifth percentile, and weight was at the tenth percentile. Head circumference was microcephalic (below fifth percentile). She had aggressive behavior as well as temper tantrums. No clinical seizures were reported, and the electroencephalogram findings were normal. She was treated with 375 mg of sodium valproate daily, as well as risperidone and extended-release guanfacine for her behavioral symptoms.

Based on the constellation of symptoms in the sibling pair, there was concern for an underlying genetic etiology. Chromosomal microarray analysis was performed to look for microduplications and microdeletions, which showed biallelic deletions in the neurexin 1 gene (*NRXN1*). This gene is associated with Pitt-Hopkins syndrome (PTHS) (OMIM: 610954). This syndrome is classically characterized by severe intellectual disability, typical facial features, a tendency for epilepsy, intermittent hyperventilation (panting-and-holding breathing anomaly), stereotypic movements, constipation, and high myopia. Growth is normal or mildly retarded, but half of the patients have postnatal microcephaly. PTHS may be caused by rare autosomal dominant mutations of *TCF4*, a member of the superfamily of basic helix-loop-helix domain of helix-loop-helix transcription factors involved in nervous and immune system development.[1,2] *NRXN1* and *CNTAP2* mutations have been associated with autosomal recessive PTHS.

Genetic Studies

Chromosomal microarray analysis was performed in our patients to identify copy number variations associated with an underlying genetic etiology.

The studies were approved by the institutional review board of St. Christopher's Hospital. The patients had normal results for an extensive range of tests, including a detailed metabolic investigation and routine karyotyping. Genomic DNA was isolated from blood for whole-genome array single nucleotide polymorphism comparative genomic hybridization. Genomic analysis was performed using the UCSC genome and Ensemble Browsers Array. Comparative genomic