Novel Homozygous Deletion in *STRADA* Gene Associated with Polyhydramnios, Megalencephaly, and Epilepsy in Two Siblings: Implications for Diagnosis and Treatment

Novel STRADA Mutation in Two Siblings

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Abstract

Mutations in the STE20-related kinase adaptor α (*STRADA*) gene have been reported to cause an autosomal recessive neurodevelopmental disorder characterized by infantile-onset epilepsy, developmental delay, and cranio-facial dysmorphisms. To date, there have been 17 reported individuals diagnosed with STRADA mutations, 16 of which are from a single Old Order Mennonite cohort and share a deletion of exons 9-13. The remaining individual is of consanguineous Indian descent and has a homozygous single base pair duplication. We report a novel *STRADA* gene deletion of exons 7-9 in two sisters from non-consanguineous parents, as well as an improvement in seizure control in one sibling following treatment with sirolimus, an m-Tor inhibitor of potential benefit to patients with this genetic mutation.

Keywords

Children, epileptic encephalopathy, genetics, neonatal seizures, neurodevelopment, next-generation sequencing, seizures

Introduction

The STRADA gene, located on 17q23.3, contains 13 exons and encodes STE20-related kinase adaptor protein. It is translated into the pseudokinase protein STE20-related kinase adapter protein α (STRADα/STRADA), which is an upstream inhibitor of the mammalian target of rapamycin complex 1 (mTORC1). [1] Mutations in STRADA have been associated with a rare clinical neurodevelopmental syndrome,first described in a cohort of 16 Old Order Mennonite patients, all of whom shared a terminal deletion in the STRADA gene and whose disorder was termed Polyhydramnios, Megalencephaly, and Symptomatic Epilepsy Syndrome (PMSE). [2] An additional patient of consanguineous parents with a similar phenotype was subsequently found to have a single base pair duplication in the same gene, causing a translational frameshift and premature termination. [3] Mutations in STRADA have been shown to cause activation of the mTOR pathway, potentially indicating a therapeutic target for mTOR inhibiting medications. [4] We describe two sisters of non-consanguineous parents with a novel pathogenic STRADA mutation and their response to treatment with sirolimus, an mTOR inhibitor.

Clinical report

Patient A, the older sibling, is a 16 year old girl of Caucasian descent born at 34+4 weeks gestation following a pregnancy complicated by polyhydramnios. She was noted to have mild

epicanthal folds, prominent nasolabial folds, full lips, and macrocephaly (Figure 1A). As an infant she was hypotonic and had poor weight gain.

[Insert Figure 1]

Global developmental delays were present throughout. She did not sit independently until age 4, and she began to stand at age 9. She has remained non-verbal and non-ambulatory to date. She developed stereotyped and repetitive behaviors, and was diagnosed with autism spectrum disorder. Obstructive sleep apnea was present as an infant, and at age three she was diagnosed with nephrocalcinosis and nephrolithiasis, requiring nephrolithotomy. Hypothyroidism was later diagnosed at age 10.

Radiographically she was found to have hypoplastic distal phalanges in all toes, mild hypoplasia of the distal first phalange on one hand, and mild pectus carinatum. Magnetic resonance imaging (MRI) at nine months of age showed moderate-to-severe diffuse white matter volume loss, and the most recent MRI at age 15 showed additionally mild cerebellar volume loss, focal encephalomalacia of the left middle temporal gyrus, and severe foramen magnum stenosis due to due to the presence of os odontoideum and ligamentous laxity (Figure 2A and 2B). Muscle biopsy identified a deficiency in complex 1, leading to an initial diagnosis of possible mitochondrial cytopathy.

[Insert figure 2]

At 4 months of age, the patient developed tonic motor seizures of unknown onset with duration of 30 to 60 seconds, and was initially treated with phenobarbital with good response. At 6 months of age, the patient developed infantile spasms, which were confirmed on electroencephalography demonstrating an ictal pattern of high amplitude sharp wave discharges on a hypsarrhythmic background. Infantile spasms initially remitted following treatment with adrenocorticotropic hormone (ACTH), but would recur around one year of age. Throughout her childhood, she continued to have refractory focal and bilateral clonic seizures, with multiple seizures daily and periodic status epilepticus. Her epilepsy would prove refractory to multiple anticonvulsants.

Patient B, the younger sister, is currently 13 years old. She was born at 38 weeks gestation following a pregnancy also complicated by mild polyhydramnios. She had facial dysmorphism similar to her older affected sibling (figure 1B). Neonatally she had feeding dysfunction and aspiration requiring gastrostomy tube placement.

A post-natal renal ultrasound was normal, but a follow-up study at 7 months of age revealed nephrocalcinosis, which would remain largely stable until her most recent renal ultrasound at age 13. She did not develop hydronephrosis or frank nephrolithiasis. Her first brain MRI at 4 months of age showed periventricular leukomalacia and subsequent imaging has shown a stable moderate degree of cerebral white matter volume loss and a few small foci of

nonspecific gliosis in the frontal and parietal subcortical white matter (Figure 2C and 2D). Sleep study at age 5 showed severe central sleep apnea.

In regards to her epilepsy, she first had a left focal tonic seizure at 4 months of age, and EEG at that time demonstrated left temporal-occipital sharps. She was treated with phenobarbital. By 8 months of age she developed infantile spasms associated with a hypsarrthymic EEG. These responded to ACTH therapy, and all seizures remitted until age 5 while she was maintained on lamotrigine. At that time she had onset of focal motor seizures with impaired consciousness, and her EEG evolved to show severe diffuse slowing and multifocal sharps, most prominent in the bitemporal areas. Her seizures would prove refractory to multiple anticonvulsants, and she experienced multiple bouts of status epilepticus, until age 10. At that time she was started on valproic acid, and she had a period of seizure remission for several years before they ultimately returned and would subsequently prove medically refractory to date.

Materials and Methods

Whole exome sequencing was performed through GeneDx. Patient A and parental serum samples were obtained and tested using XomeDxPlus, which combines whole exome sequencing with mitochondrial genome sequencing and deletion testing. The patient's sample was run on the Agilent Clinical Research Exome Kit. Exonic regions and flanking intronic splice junctions were sequenced simultaneously by massively parallel (NextGen) sequencing on an Illumina HiSeq 2000 instrument. 100 base pair reads were bidirectionally sequenced, assembled, and aligned to the GRCh37/UCSC hg19 reference sequence. Sequences were

analyzed for variants using Xome Analyzer analysis tool. Capillary sequencing was used to confirm pathogenic variants.

Results

Exome sequencing of patient A identified a novel homozygous 4kb deletion in the *STRADA* gene of exons 7-9 at chr17:61,780,815-61,784,837, respectively. GeneDx clinical test XomeDxPlus was utilized for the patient's genetic testing. This deletion is at the 3 prime end of the *STRADA* gene in the protein kinase domain. Functional studies have not been done to verify RNA transcript or protein function. Only loss of function mutations have been reported associated with STRADA, and it is likely this deletion also causes loss of function. [3] Parental samples from the exome trio showed bilineal inheritance of a heterozygous deletion of exons 7-9 of the *STRADA* gene. Polymerase chain reaction (PCR) analysis through GeneDx was done for patient B which revealed an identical homozygous deletion.

Intervention

Sirolimus binds to FKBP-12, an intracellular protein, to form an immunosuppressive complex which inhibits the regulatory kinase, mTOR.[5] Based on previous studies suggesting benefit in regards to epilepsy following treatment with an m-Tor inhibitor, both sisters were treated with sirolimus oral solution at 0.5 mg/m^2/dose once daily. [6] Doses were adjusted on a weekly basis initially to achieve a target trough level of 5–15 ng/mL. Of note, Patient B was on phenobarbital, which

is a known CYP3A4 inducer. After one year of sirolimus therapy, the family reported that Patient A's seizure frequency has decreased significantly. Where previously she was having multiple daily seizures, after treatment her seizure frequency decreased to where she would have days without seizures, and could go as long as 5 days without a seizure. This decrease was quantified in a seizure log maintained by her parents, which unfortunately was not started until 3 months after beginning therapy (Figure 3). Her parents also reported subjective improvement in her behavior, attention, and a decrease in somnolence.

[Insert figure 3]

Patient B, however, continued to have many seizures per day. Her sirolimus trough levels remained low despite increasing her dose, likely due to co-commitant phenobarbital therapy. Attempts were made to wean phenobarbital in the hope of increasing her sirolumus levels, which was not tolerated due to an increase in seizure burden. She did show some mild, subjective improvements in alertness, but had no other obvious benefits to therapy. Similar to that reported in the Mennonite children, no obvious effects on expressive language, gross motor function, or adaptive learning were reported in either sibling. [1] Sirolimus was tolerated well, the only side effect being hypertriglyceridemia, which was treated with finofibrate.

Discussion

This novel *STRADA* exon 7-9 deletion, in addition to the previously described case of a 5-year-old boy with a single base duplication, demonstrates that pathogenic changes in this gene occur

outside of the Old Order Mennonite community. Despite homozygosity for an exon 7-9 deletion in the *STRADA* gene, there is no reported or clinically suspected consanguinity in this case. While we cannot discount the possibility of the parents being distantly related beyond the known family history, homozygosity for a rare gene mutation in a suspected rare disorder can also be explained by potential mutational hotspots or coincidentally occurring mutations. Either mechanism implies that *STRADA* mutations may not be limited to restricted populations and may be an under-recognized in children with infantile epileptic encephalopathies. While several of these syndromes are clinically distinct, they may be difficult to differentiate phenotypically. Accurate diagnosis is important as prognosis, recurrence risk, and potential for treatment may vary. STRADA-related disorders may be one of a relatively small number of these disorders for which treatment of the underlying defect has shown promise, although further study is needed to establish the efficacy of m-Tor inhibitors in these disorders.

Given that our patients' phenotype is severe and similar to that described in the Mennonite population, it is possible that they have a similar biochemical phenotype that would also respond to sirolimus therapy, although our patients differed from that previously reported in several ways. First, their mutation differed from that described in the Mennonite population, which may cause different functional effects on the STRADA protein and the mTORC1 pathway. Secondly, our subjects were significantly older at the time of treatment, so it is possible earlier treatment would have resulted in a more robust response. Ultimately, patient A seemed to show a sustained improvement in seizure control, while no clear effect was seen in patient B.

STRADA mutations are associated with a severe early-onset childhood epilepsy phenotype. While these children have several features that may differentiate their disorder from other severe childhood epilepsies, such as nephrocalcinosis and megaloencephaly, the phenotype may otherwise overlap significantly with other infantile epileptic encephalopathies. Our cases demonstrate that this disorder occurs outside of a sequestered population, and suggests these disorders have a potential specific therapy. We propose that early identification of STRADA-associated disorders allows more effective surveillance, anticipatory guidance, and potential treatment for affected children. We also propose that *STRADA* mutation analysis be added to the increasingly comprehensive next-generation sequencing panels now available for infantile and childhood epilepsies.

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Author Contribution

KN was primary in drafting and finalizing the manuscript. CJ, LW, CS, JB contributed significantly to the body of the manuscript, and CJ did the majority of revisions for resubmission. LW, KP and SD provided revisions and guidance throughout the drafting process. LW, CS, JB and KP all participated in the clinical care of the patients.

Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research authorship and/or publication of this article.

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Ethical approval

Photography consent forms were obtained from the patients guardians.

Figure 1A and 1B: Both sisters (patient A being the elder) affected by the same STRADA mutation showed similar dysmorphic features

Figure 2: Axial FLAIR MRI images from Patient A showing focal encephalomalacia in the left temporal lobe (2A). Patient A (2B) and Patient B (2C and 2D) demonstrated a decrease in white matter volume with punctate T2 white matter hyperintensities

Figure 3: Seizure log demonstrating decrease in seizure burden for patient A following sirolimus treatment