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REVIEW

The expanding phenotypic spectrum of female *SLC9A6* mutation carriers: a case series and review of the literature

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Abstract Christianson syndrome (OMIM 300243), caused by mutations in the X-linked *SLC9A6* gene, is characterized by severe global developmental delay and intellectual disability, developmental regression, epilepsy, microcephaly and impaired ocular movements. It shares many common features with Angelman syndrome. Carrier females have been described as having learning difficulties with mild to moderate intellectual disability, behavioural issues and psychiatric illnesses. There is little literature on the carrier female phenotype of Christianson syndrome. We describe a large extended family with three affected males, four carrier females, one presumed carrier female and one obligate carrier female with a c.190G>T, p.E64X mutation known to cause a premature stop codon in *SLC9A6*. We characterize and expand the clinical phenotype of female *SLC9A6* mutation carriers by comparing our described family with female carriers previously discussed in the literature. In particular, we highlight the neurodevelopmental and psychiatric phenotypes observed in our family and previous reports.

Introduction

Christianson et al. (1999) first described a five-generation, Caucasian South African family with multiple affected males presenting with profound mental retardation, epilepsy, mild craniofacial dysmorphism, bilateral ophthalmoplegia, truncal ataxia and limited life expectancy. This syndrome had many characteristic features overlapping with Angelman syndrome (OMIM105830), including developmental delay, acquired microcephaly and happy demeanour. The syndrome, now referred to as Christianson syndrome (CS), was found to be caused by mutations in *SLC9A6* (Gilfillan et al. 2008). The *SLC9A6* gene encodes for the sodium/hydrogen exchanger NHE6. NHE6 is highly expressed in the brain, and is thought to participate in the targeting of intracellular vesicles and recycling of synaptic vesicles (Garbern et al. 2010). The Na+/H+ exchanger gene family has been linked to autism and neurologic disease (Kondapalli et al. 2014).

Descriptions of female carriers of *SLC9A6* mutations have been infrequent. Reported phenotypic findings of carrier females include learning problems, mild to moderate intellectual disability, behavioural problems and psychiatric illnesses (Schroer et al. 2010; Pescosolido et al. 2014).

We present a large extended family of Caucasian background with three affected males with CS, four confirmed *SLC9A6* mutation carrier females, one obligate carrier female and one presumed carrier female. We characterize the carrier females in our family based on clinical features, developmental history and psychiatric disorders, and compare them to the available literature on other *SLC9A6* carrier females.

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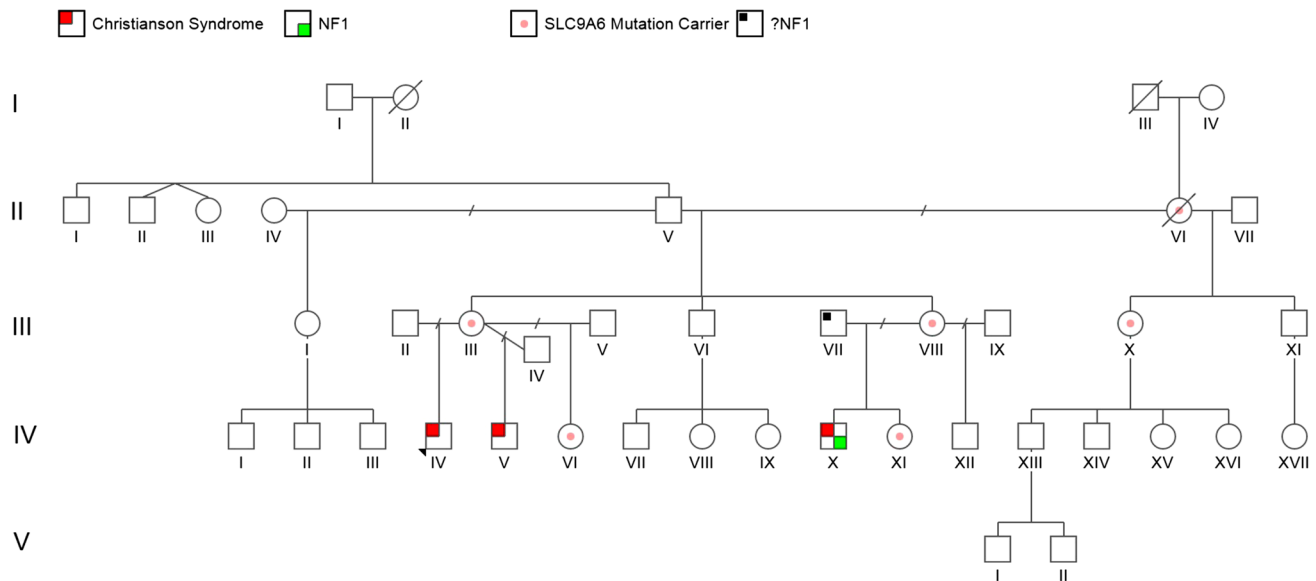
Fig. 1 Extended pedigree of family with three affected males with Christianson syndrome, four *SLC9A6* mutation carrier females, one assumed carrier (IV–XI) and one obligate carrier (II–VI)

Materials and methods

All male and female family members reported in this study were clinically assessed by at least one medical geneticist. A detailed chart review was also undertaken for consultation reports from other specialists, including developmental, neurological and psychiatric assessments, where available.

Genomic microarray analysis was performed using the 4 × 180 K Oligonucleotide Array (Agilent Technologies). Testing for Fragile X syndrome was performed by PCR amplification using the Asuragen AmplideX *FMR1* PCR kit to detect CGG repeat size in the 5′ UTR of the *FMR1* gene, and by PCR amplification and Southern blot analysis with methylation-sensitive enzymes to detect CCG repeat size and methylation status of the *FMR2* gene. Testing for Prader–Willi syndrome was performed by multiplex ligation-dependent probe amplification to determine the methylation status at the *SNRPN* locus and gene dosage of the *SNRPN* gene. Testing for mutations in the *NF1* and *SPRED1* genes associated with neurofibromatosis type 1 (NF1) and Legius syndrome, respectively, was performed by Sanger sequencing.

Sequencing of *SLC9A6* was performed as part of an X-linked mental retardation next-generation sequencing (NGS) panel (Ambry Genetics, CA, USA). In brief, genomic DNA was isolated from the patient's specimen using a standardized kit and quantified by agarose gel electrophoresis. Sequencing was carried out using PCR and

NGS using primer pairs designed to target the 81 genes on the panel. Additional Sanger sequencing was performed for any regions missing coverage and regions with sequence similarity elsewhere in the genome. Targeted Sanger sequencing was performed in additional family members after an *SLC9A6* mutation was detected in the index case.

Results

A disease-causing mutation was found in exon 1 of the *SLC9A6* gene (c.190G>T, p.E64X). The G to T substitution at nucleotide position 190 causes an amino acid change from glutamic acid to a stop codon within exon 1. The mutation was first detected in individual IV–IV (Fig. 1).

Clinical report

Males affected with CS

Patient IV–IV (Index Case)

IV–IV is a 21-year-old male with global developmental delay, seizure disorder with onset at 1.5 years of age and autism spectrum disorder diagnosed at 15 years of age. Genetics assessment at 18 years of age showed a head circumference of 55 cm (47th percentile, −0.07 SD). He had a history of serious infection with influenza B myositis and encephalitis.

Patient IV–V

IV–V is an 18-year-old male with early childhood microcephaly, hearing loss, global developmental delay and seizure disorder. He was able to achieve some developmental milestones, including walking at 3 years of age. He suffered acquired brain damage at 5 years of age with accidental self-strangulation with subsequent regression in all spheres and is severely delayed. Genetics assessment at 16 years of age showed a head circumference of 51.5 cm (1st percentile, −2.42 SD).

Patient IV–X

IV–X is a 12-year-old male with severe developmental delay and seizures beginning at under 1 year of age. At genetics assessment at 10.5 years of age, he had a head circumference of 52 cm (21st percentile, −0.79 SD). He also has multiple neurocutaneous stigmata, including café-au-lait spots, extensive inguinal and axillary freckling, and neurofibromas. He was molecularly confirmed to be affected with NF1 with heterozygous deletion of exons 10–30 of the *NF1* gene.

Female *SLC9A6* mutation carriers

Table 1 summarizes the neurodevelopmental, psychiatric and neurologic findings of the female *SLC9A6* mutation carriers in this family, along with their genetic testing results.

Patient II–VI

II–VI presented at 60 years of age with an akinetic right arm with apraxia, cortical sensory loss, worsening emotional lability, myoclonus and slow saccades with saccadic dysmetria. Initial neurologic assessment showed impaired fine motor control, spasticity in the upper extremities bilaterally, along with marked saccadic smooth pursuit. Vertical gaze was intact. Two years later, prior to her death, she had marked supranuclear gaze palsy and paralysis of the right side. Neurological opinion favoured a taupathy that fit within the corticobasal degeneration syndrome (CBDS) and progressive supranuclear palsy spectrum, with findings most compatible with CBDS. She had cognitive testing with Mini Mental Status Exam (Folstein et al. 1975) score of 22/30 indicating early cognitive decline, and Behaviour Neurology Assessment-Short Form (Darvesh et al. 2005) score of 48/114 with deficits in all cognitive domains. Initial MRI showed diffuse cortical and cerebellar atrophy. A second MRI with contrast showed a striking asymmetric pattern of atrophy, most prominent in the left frontal and parietal cortex, in keeping with CBDS. She had progression of the disease over time and multiple falls, and passed away at 64 years of age due to aspiration pneumonia. She did not undergo testing for the familial *SLC9A6* mutation, but is an obligate carrier based on the test results of her offspring.

Patient III–III

III–III is a 38-year-old female with anxiety and depression treated with citalopram, learning difficulties and a history of self-cutting behaviour. She was able to complete grade 9 at an appropriate age but did not complete grade 12 until she was 38 years of age. At psychiatric assessment at age 38 years, she had moderate psychomotor retardation, was coherent and logical with slow mentation, and was cognitively grossly intact. She was diagnosed with major depressive disorder, anxiety disorder with symptoms of generalized anxiety disorder and panic disorder, along with psychosis not otherwise specified (NOS), possibly related to her underlying genetic condition. At genetics assessment at 38 years of age, she was apparently normocephalic, non-dysmorphic and had no obvious neurologic deficits.

Patient III–VIII

III–VIII is a 34-year-old female with a history of learning difficulties in school, seizure disorder and psychiatric diagnoses. Her seizure disorder began with grand mal seizures in childhood that have evolved to occasional focal seizures around the eyes. These were described to be triggered by smells or spices. Psychiatric assessment at 33 years of age showed her to be cognitively intact with average intelligence, coherent and logical. She was given DSM-IV diagnoses of dysthymic disorder (with query for bipolar disorder type II), anxiety disorder NOS with symptoms of generalized anxiety disorder, panic disorder and post-traumatic stress disorder-like symptoms, some cluster B personality traits, impulsivity, interpersonal difficulties and affect dysregulation. She was found to be moderately impaired with a Global Assessment of Functioning (GAF) (Jones et al. 1995) score of 55. At genetics assessment at 34 years of age, she was apparently normocephalic, non-dysmorphic and had no obvious neurologic deficits. She had normal microarray testing.

Patient III–X

III–X is a 49-year-old female with a history of learning difficulties in school. She required extra help in all subjects and recently completed grade 12 at the age of 38. She had normal early development. She had behavioural issues in early childhood presenting as nocturnal enuresis. She was diagnosed with depression in her 40s, which has been treated with citalopram and quetiapine. Psychiatric

Table 1 Phenotypic findings of reported female *SLC9A6* mutation carriers compared to cases in the literature

Patient	Age (years)	Development			Psychiatric diagnoses				Neurologic		Genetic test results
		Speech/language delay/difficulties	Early developmental delay	Intellectual disability	Mood disorders	Anxiety disorders	Behavioural disorders	Psychotic disorders	Neurologic disorders	Brain imaging	
II–VI	65								Corticobasal degeneration	MRI: asymmetric atrophy most prominent in left frontal and parietal cortex	Obligate carrier of *SLC9A6*—c.190G>T, p.E64X
III–III	38		–	+	+	+	+	+			*SLC9A6*—c.190G>T, p.E64X
III–VIII	34		–	+	+	+	+		Seizure disorder		GMA—normal *SLC9A6*—c.190G>T, p.E64X
III–X	49		–	+	+	+	+				GMA—normal Fragile X—normal *SLC9A6*—c.190G>T, p.E64X
IV–VI	16	+		+		+	+	+			Karyotype—normal Fragile X—normal *SLC9A6*—c.190G>T, p.E64X NF1—Normal *SPRED1*—normal
IV–XI	9	+		+	+		+				GMA—normal Fragile X—normal PWS—normal *SLC9A6*—declined

Table 1 continued

Patient	Age (years)	Development		Intellectual disability	Psychiatric diagnoses				Neurologic		Genetic test results
		Speech/language delay/difficulties	Early developmental mental delay		Mood disorders	Anxiety disorders	Behavioural disorders	Psychotic disorders	Neurologic disorders	Brain imaging	
Paediatric carrier females in the literature (Christianson et al. 1999; Schroer et al. 2010; Pescosolido et al. 2014)		+ (1/6)		+ (6/6)	+ (1/6)	+ (1/6)	+ (1/6)	+ (2/6)	Hyperkinesis (Schroer et al. 2010) Mild truncal ataxia (Schroer et al. 2010)		1/6—Detected through linkage analysis (Christianson et al. 1999) 5/6—Confirmed *SLC9A6* mutation (Schroer et al. 2010; Pescosolido et al. 2014)
Adult carrier females in the literature (Christianson et al. 1999; Schroer et al. 2010; Riess et al. 2013; Zanni et al. 2014; Fichou et al. 2009; Gilfillan et al. 2008)		+ (2/20)	+ (1/20)	+ (4/20)	+ (1/20)		+ (1/20)	+ (2/20)	2 cases of Parkinson's disease (Zanni et al. 2014)		10/20—Obligate carriers (Christianson et al. 1999; Schroer et al. 2010; Zanni et al. 2014; Fichou et al. 2009) 8/20—Confirmed *SLC9A6* mutation (Riess et al. 2013; Zanni et al. 2014; Gilfillan et al. 2008) 2/20—Unclear testing (Zanni et al. 2014; Fichou et al. 2009)

GMA genomic microarray analysis, *PWS* Prader–Willi syndrome testing

assessment at 45 years of age showed intact cognition but poor memory and concentration. She was given DSM-IV diagnoses of chronic major depression (most likely in the phase of dysthymia), panic attacks and agoraphobia. She had a GAF (Jones et al. 1995) score of 52. At genetics assessment at 48 years of age, she was normocephalic with a head circumference of 54 cm (38th percentile, −0.30 SD), non-dysmorphic and had a normal neurologic examination. She had normal microarray and Fragile X syndrome testing.

Patient IV–VI

IV–VI is a 16-year-old female with developmental delays starting at 17 months of age in cognitive and fine motor skills. She had episodes of selective mutism that began at 4 years of age, along with speech difficulties. She has received speech therapy since 4 years of age. Because of difficulties in traditional school arrangements, she has required special classes with smaller numbers of students. She has been diagnosed with attention deficit hyperactivity disorder (ADHD), separation anxiety, oppositional defiant disorder (ODD) manifesting as violent tantrums and physical aggression, and possible schizophrenia. At genetics assessment at 13.5 years of age, she was normocephalic with a head circumference of 52.6 cm (15th percentile, −1.04 SD) and had a normal neurologic examination. She has multiple café-au-lait spots, and inguinal and axillary freckling, giving her a clinical diagnosis of NF1. She had normal ophthalmological assessments and a reportedly normal brain MRI. She had normal microarray and Fragile X syndrome testing, as well as normal testing for the *NF1* and *SPRED1* genes.

Patient IV–XI

IV–XI is a 9-year-old female with delays in speech development. Babbling began at 15 months and her first comprehensible words appeared at 3 years of age. At age 7 years, she was in grade 1 with extra help required for speech and language. Neuropsychological testing demonstrated good math skills and very poor language skills. Behavioural issues started at 1.5 to 2 years of age with temper tantrums. She has been diagnosed with ADHD and ODD, and a possible mood disorder. She has ongoing sleep difficulties and self-injurious behaviours, including head-banging and self-induced vomiting. At genetics assessment at 7.5 years of age, she had a head circumference of 54.7 cm (>97th percentile, +2.41 SD), with normal neurologic examination. She had normal microarray analysis, methylation studies for Prader–Willi syndrome and Fragile X syndrome testing. The family declined to have IV–XI undergo targeted mutation testing for the familial *SLC9A6* mutation.

Discussion

CS was first described in 1999 in a single family with an initial cohort of 16 affected males and 10 carrier females over five generations. The initial ten carrier females (with eight obligate carriers and two carriers confirmed by linkage analysis) were briefly described with a wide clinical spectrum, ranging from mentally and physically normal in six females to two females who were mildly affected with behavioural problems and aggression during childhood (Christianson et al. 1999). The female *SLC9A6* mutation carrier phenotype has since been expanded to include learning difficulties in school, speech and language delay, intellectual disability ranging from mild to moderate, and behavioural issues, most notably aggression (Christianson et al. 1999; Schroer et al. 2010; Pescosolido et al. 2014). In general, the female phenotype shows no dysmorphic features, no microcephaly [one described patient with microcephaly (−3 SD) (Pescosolido et al. 2014)] and no seizures.

Table 1 summarizes the neurodevelopmental, psychiatric and neurologic findings of the females reported in our study compared to those previously reported in the literature. In our female cohort, there is an apparent overall decline in academic functioning in subsequent generations, along with more prevalent speech and language difficulties, and increased behavioural issues. In generation III, individuals III, VIII and X all reported learning issues in school, most prevalent in higher grades with difficulty in completing high school; III–III and III–X were able to complete high school-level courses as adults. No females in this third generation had issues with early development, speech or language. Their early behavioural concerns consisted of self-injurious behaviours (e.g. cutting) and nocturnal enuresis.

The female mutation carriers in the fourth generation of the family were all more severely affected after a period of normal early development. Subsequent developmental issues were generally noted to start between ages 2 and 4 years. Both IV–VI and IV–XI required speech therapy at an early age (3 and 5 years, respectively), and their learning difficulties began much earlier. With traditional classroom arrangements being too difficult, they have required special class arrangements since entering grade 1 and individualized education plans. Behavioural issues also began in early childhood, with ODD that manifested as aggression towards family, self-injurious behaviours and constant temper tantrums. The apparent progressively earlier presentation in subsequent generations has not been characterized before in the female phenotype. Modern surveillance with increased testing, vigilance, and awareness of early developmental and behavioural disorders may create an ascertainment bias when characterizing generational differences.

Nevertheless, recognizing the potential for variable, and possibly more severe, expression in subsequent generations would be beneficial in assessment of large affected families to allow for earlier interventions.

In the family reported here, there was a higher frequency and variety of psychiatric disorders than previously described in the female *SCL9A6* mutation carrier phenotype. The most common psychiatric disorders in female *SLC9A6* mutation carriers in this family were mood, behavioural and anxiety disorders, including major depression, ADHD, ODD, generalized anxiety, panic disorder and agoraphobia. Several had symptoms suggestive of psychotic disorders. The prevalence of psychiatric disorders in adults with *SLC9A6* mutations has not previously been described extensively in the literature. Riess et al. (2013) described one obligate carrier female who was healthy until 55 years, when she developed neurologic symptoms and depression. An ascertainment bias may be present in our family given the complexity of family dynamics and concomitant medical issues. However, there is a clear trend indicating that psychiatric manifestations are likely an underdiagnosed manifestation of *SLC9A6* mutation carrier status that warrants closer attention. Furthermore, a similar generational phenomenon as seen with neurodevelopmental and behavioural disorders was also observed with psychiatric findings, with earlier and more severe psychiatric impairment present in subsequent generations of female mutation carriers. Those in the fourth generation showed much earlier ages of onset and a wider spectrum of psychiatric disorders, including mood and anxiety disorders, and more severe behavioural disorders, including ADHD, ODD and self-injurious behaviours.

Interestingly, Pescosolido et al. (2014) described a female *SLC9A6* mutation carrier with a strikingly similar phenotype to patient IV–VI in our study. This individual (no age stated) was described to have moderate intellectual disability, speech and language delay, selective mutism, ODD, reactive attachment disorder and ADHD (Pescosolido et al. 2014). Unfortunately, there was no description of the *SLC9A6* mutation for this patient to allow for potential genotype–phenotype correlations. Nevertheless, the similar clinical spectrums of these two patients underscore the emerging complex phenotypic picture of female *SLC9A6* mutation carriers.

In our study, there were several confounding medical and genetic factors that may have influenced the ultimate phenotypes seen. One female (III–VIII) has a long-standing seizure disorder, the aetiology of which is unclear. No female mutation carriers have previously been reported with seizure disorders and it remains unclear whether this may be attributable to carrier status. Another female (IV–VI) met clinical criteria for a diagnosis of NF1 (OMIM 162200), with findings of multiple café-au-lait spots, and inguinal and axillary freckling on physical examination; molecular testing did not identify a pathogenic basis for her findings. Neurodevelopmental disorders, including learning difficulties, intellectual disability and autism spectrum disorders, are known to occur frequently in individuals with NF1 (Friedman 1998). Patient IV–XI did not undergo testing for the familial *SLC9A6* mutation, though her clinical presentation, family history and other normal genetic test results strongly suggest that she is a carrier of the familial mutation.

Patient II–VI was not tested for the familial *SLC9A6* mutation but could be determined to be an obligate carrier based on the results of testing in her offspring. Her distinct neurological findings were highly suggestive of a diagnosis of CBDS, which is one of the atypical parkinsonian disorders that can mimic the clinical presentation of classic Parkinson's disease (Fogel et al. 2014). CBDS presents with distinct pathological findings of accumulation of 4R (four repeat) tau isoforms (Grijalvo-Perez and Litvan 2014). The classical clinical presentation is of asymmetric parkinsonism, ideomotor apraxia (inability to correctly use hand gestures and mime tool use), rigidity, myoclonus and dystonia (Grijalvo-Perez and Litvan 2014). Patient II–VI presented with an akinetic right arm with apraxia, cortical sensory loss, worsening emotional lability, myoclonus and slow saccades with saccadic dysmetria. CBDS has variable phenotypic presentation, which can make diagnosis difficult; an autopsy is needed for confirmation (Grijalvo-Perez and Litvan 2014).

Riess et al. (2013) described two obligate carrier females from their affected CS family. The grandmother of the index case, an obligate carrier, was healthy until 55 years when she developed neurologic symptoms and depression. Her brain imaging showed diffuse mild cerebral atrophy and she was given a diagnosis of Parkinson's disease. Her mother was reported to have parkinsonism in her 70s and passed away at 82 years of age. Given the clinical similarity and age of onset of atypical parkinsonian disorders and Parkinson's disease, distinguishing between the different etiologies is difficult without further clinical information or an autopsy of those affected. It is possible that these cases may represent onset of clinical findings of the female phenotype that presented at a later age. Interestingly, the neurologic findings in this family, as in our cohort, were also observed to worsen with earlier age of and more severe presentation in subsequent generations. Together, these findings represent a new aspect to the female *SLC9A6* mutation carrier phenotype, with increasing evidence that females may be at risk for cognitive and neurologic decline as they age, with pathological and phenotypic features similar to CBDS or Parkinson's disease.

There is increasing evidence to suggest that these neurodegenerative findings may reflect a tauopathic process associated with *SLC9A6* functional impairment. Garbern

et al. (2010) described two males with an in-frame 9-base pair deletion in *SLC9A6* who were found to have pathologic findings of tau deposition very similar to CBDS. The two patients were reported to have profound mental retardation and severe language disturbance, similar to that seen in CS, but the dysmorphic features and severe microcephaly were not seen. They postulated that progressive neurodegeneration was caused in part by tau deposition, which contributed to the severe neurodevelopmental phenotype. Neuropathological examination showed widespread cerebral atrophy and marked neuronal loss. The cerebellar and brainstem white tracks showed diffuse glial tau pathology, while tau-positive inclusions were widespread in the cerebral cortex and hippocampus. Their observations suggested that the deposition of tau may have been mediated by the interaction with the mutant *SLC9A6* protein. They postulated that mutations in *SLC9A6* can cause aberrant splicing in the *MAPT* gene which encodes tau, leading to tau deposition.

Strømme et al. (2011) developed Slc9a6 knockout mouse models which showed that loss of function of Slc9a6 leads to altered endosomal–lysosomal function, and accumulation of GM2 gangliosides and cholesterol in select neuronal populations (including amygdala and hippocampus) similar to primary lysosomal storage diseases. They noted extensive Purkinje cell deterioration and axonal spheroid formation in the cerebellum, findings which were correlated with the cerebellar atrophy seen with human *SLC9A6* mutations. The knockout mice also showed small increases of phosphorylated tau in soluble brain fractions which could be correlated with the behaviour of the knockout mice. Strømme et al. (2011) postulated that the specific areas involved could provide a unified explanation for the phenotypes seen. The findings of tau deposition as a downstream consequence of endosomal–lysosomal dysfunction (Strømme et al. 2011), along with the hypothesis that this deposition may be further provoked by the interaction of a mutant *SLC9A6* protein (Garbern et al. 2010), raise interesting questions about the role of tau deposition in both the male and female phenotypes associated with *SLC9A6* mutations.

As with many X-linked disorders, skewed X-inactivation may play a role in the clinical presentation of *SLC9A6* mutations in female carriers. Sikora et al. (2016) created a heterozygous Slc9a6 knockout female mouse that showed the expression of the mutant Slc9a6 allele to follow the same neuroanatomic distribution and cell-specific patterns in hemizygous mutant males. The heterozygous knockout female mice developed progressive behavioural and neuropathological abnormalities that were similar to but milder than hemizygous male knockout mice. Similar to Strømme et al. (2011), they showed accumulation of GM2 gangliosides in the hippocampus and amygdala, along

with Purkinje cell degeneration in the cerebellum. Using β-galactosidase as a marker for cellular expression of the mutant Slc9a6, they showed that X-inactivation was a likely random event in Purkinje cells. They concluded that further studies should be undertaken to determine neuroanatomic thresholds for behaviour pathologies (Sikora et al. 2016).

The emerging and expanding phenotypic spectrum of female *SLC9A6* mutation carriers is reminiscent of the ever-expanding phenotypic spectrum associated with the Fragile X syndrome gene *FMR1*. Mutations in *FMR1* are associated with Fragile X syndrome (OMIM 300624), while the premutation state can result in increased risk of Fragile X-associated tremor-ataxia syndrome (FXTAS) (OMIM 300623), as well as premature ovarian failure in females. The *FMR1* narrative also began with the assumption that female carriers would have little to no susceptibility to the associated phenotypes. The less frequent, later onset and milder findings of FXTAS in female premutation carriers were thought to arise due to X-inactivation providing a protective mechanism, though this has not been fully proven (Grigsby et al. 2014). Further delving into the phenotype for female *FMR1* premutation carriers has shown progressive cognitive impairment in executive functioning, working memory and information processing (Grigsby et al. 2014). Testing of neuropsychiatric functioning in females with a premutation has shown higher rates of depression and social anxiety (Kraan et al. 2013), as well as other psychiatric disorders, such as ADHD (Hunter et al. 2012). Loesch et al. (2015) showed that carriers of mid-sized *FMR1* 5′ UTR repeats (<100 CGG repeats) are at greatest risk for developing a psychiatric disorder. It is becoming clear that female *FMR1* premutation carriers have a distinct clinical phenotype with progressive neurocognitive decline and increased risk for neuropsychiatric impairment. A similar pattern is now emerging with female *SLC9A6* mutation carriers.

While the phenomenon of anticipation is most frequently associated with disorders involving expansion of triplet repeats, there are X-linked genetic syndromes not involving repeat expansions in which anticipation has been reported. X-linked dominant chondrodysplasia punctata (OMIM 302960) is a rare skeletal dysplasia that presents with stippled epiphyses, short stature and skin lesions. It is presumed lethal in males but presents variably in females. Females can be stillborn to mildly affected in adulthood. Traupe et al. (1992) described three families with variable presentations in affected family members with increasing severity in subsequent generations. They hypothesized a heritable unstable premutation found through exclusion mapping as the cause of this phenomenon. Sutphen et al. (1995) described a three-generation family affected with X-linked dominant chondrodysplasia punctata with increasing severity of presentation in subsequent

generations. Skewed X-inactivation was hypothesized but not tested. Shirahama et al. (2003) described a family with marked variation and severity between mother and daughter which was attributed to confirmed skewed X-inactivation. Whether the observed increasing phenotypic severity can be attributable to variable skewed X-inactivation or the hypothesis of a heritable unstable premutation as proposed by Traupe et al. (1992) remains to be proven. Other X-linked conditions described with apparent anticipation attributable to skewed X-inactivation include dyskeratosis congenita (Devriendt et al. 1997), Wiskott–Aldrich Syndrome (Parolini et al. 1998) and X-linked myotubular myopathy (Tanner et al. 1999). Further studies, including in animal models, of these disorders are necessary to determine whether skewed X-inactivation provides an adequate explanation for the apparent anticipatory phenomenon seen in female carriers of non-triplet repeat X-linked disorders. Further longitudinal studies of human female *SLC9A6* mutation carriers will also be essential to better characterize the neurodegenerative phenotype and determine the relative risk for developing these symptoms. X-inactivation studies may be of value for female mutation carriers to determine their risk for neurodegeneration. Neurologic and psychiatric assessments with periodic follow-up may be warranted to detect subtle neurologic findings or developing psychiatric illness.

The female *SLC9A6* mutation carrier phenotypic spectrum encompasses learning difficulties, speech and language delay, mild to moderate intellectual disability, and behavioural issues. The results of this study provide further support for these phenotypes and expand the phenotype to suggest a significant risk for a variety of psychiatric disorders, including mood and anxiety disorders, ADHD, ODD, and disorders that can involve psychosis, such as bipolar disorder and schizophrenia. Some of these disorders had been previously infrequently reported. The most prominent behavioural phenotypes seen are aggression and self-injury. There is also increasing evidence of a neurodegenerative component to the female carrier phenotype, with this and other reports of CBDS and parkinsonian disorders, and the possibility of *SLC9A6* mutations being involved in a tauopathic process. Finally, empiric evidence from this study and the literature suggests an anticipatory element, with worsening of the female carrier phenotype in subsequent generations. This would have important implications in the counselling and management of families in which multiple generations have been identified to carry a mutation in *SLC9A6*. It remains unclear whether the emerging findings constitute a distinct female phenotype for *SLC9A6* mutations or whether they are more akin to the *FMR1* premutation status. There is currently no known correlation between X-inactivation status and phenotype (Christianson et al. 1999; Gilfillan et al. 2008; Riess et al. 2013),

but further studies on larger cohorts may aid in elucidating other pathomechanisms for the phenotypic findings. In the meantime, the growing body of evidence for a female phenotype supports that description of "affected males" and "carrier females" would be more accurately represented by describing individuals as simply being affected or not with a mutation in *SLC9A6*.

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