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EXONIC DELETION OF *SLC9A9* IN AUTISM WITH EPILEPSY

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Genes encoding proteins critical for intracellular vesicular transport are an emerging area of importance for neurologists. In particular, proteins that create and maintain the correct compartmental pH, such as the endosomal Na^+/H^+ exchangers (NHEs), have been implicated in a wide range of human diseases, including cardiovascular, inflammatory bowel, renal, and neurologic disorders, which demonstrates the critical cellular function of these proteins.[1–3] Two NHEs, NHE6 and NHE9, have been linked to neurologic disorders in children.[4] Pathologic variants in *SLC9A6* encoding NHE6 cause an Angelman-like disorder called Christianson syndrome. Fewer variants have been described in *SLC9A9* encoding NHE9, but individuals carrying these variants have been diagnosed with neurologic disorders ranging from autism to epilepsy to attention-deficit/hyperactivity disorder. The majority of described variants are missense, resulting in amino acid substitutions, making it difficult to determine their functional consequence.[4]

Case history. Our patient presented at 3 years of age with delayed language development. He was noted to have poor socialization, poor eye contact, inflexibility in new situations, and repetitive behaviors. He was administered the Autism Diagnostic Observation Schedule—Module 2 by a developmental psychologist specializing in autism when he was age 10 and was found to meet DSM-IV criteria for autistic disorder. His physical examination was notable for obesity (body mass index 31 kg/m^2), macrocephaly, and poor eye contact but normal tone.

His mother developed cryptogenic epilepsy at age 11. His father reported learning disabilities and social anxiety but no formal neurologic diagnoses. There was no consanguinity (figure, A).

At age 15, he developed focal epilepsy with secondary generalization. EEG revealed left frontotemporal epileptiform activity (figure, B). MRI was remarkable for a left anterior temporal lobe nonenhancing fluid-attenuated inversion recovery hyperintensity (figure, C). He has been seizure-free for more than 1 year on a regimen of zonisamide and levetiracetam.

Array comparative genomic hybridization using BCM Oligo V8.1 array (https://www.bcm.edu/research/medical-genetics-labs/) revealed a copy number loss of ~0.5 kb in 3q24, including exon 2 of *SLC9A9*, and a de novo copy number loss of ~0.4 kb in 2p22.3, including exon 12 of *SPG4* (figure, D). The exonic deletion of *SLC9A9* is predicted to result in an early truncation in the NHE9 protein, rendering it nonfunctional. The *SLC9A9* deletion was inherited from his father.

Discussion. NHE9 belongs to a family of intracellular cation/proton antiporters that localize to the early and recycling endosome where it alkalinizes the lumen (figure, E).[4] Alkalinization of the endosome is believed to be critical for proper cargo transport and potentially insertion of transmembrane proteins into the plasma membrane through the recycling pathway. Within the CNS, NHE9 is expressed in both neurons and astrocytes. Within astrocytes, NHE9 may have a role in glutamate uptake, with its deficiency resulting in increased synaptic glutamate and hyperexcitability of neurons.

Previously described variants in *SLC9A9* have been observed in patients with neurodevelopmental disorders with or without epilepsy. To date, a total of 7 missense mutations and 1 nonsense mutation have been described, with most individuals having both autism and epilepsy.[4] One 12-kb deletion that deletes the 5′ noncoding region of *SLC9A9* and all of a neighboring gene has also been described. Functional studies have suggested that the missense mutations result in loss of function; however, in vivo confirmation is lacking.[4]

This single case adds to the existing literature that loss-of-function mutations in *SLC9A9* cause variable neurologic disorders. Given our patient's history of a phenotype similar to those previously described with *SLC9A9* variants, this deletion potentially explains his phenotype. Of interest, he was also found to harbor a de novo deletion in *SPG4*, a cause of hereditary spastic paraplegia (HSP) predicted to cause a frameshift and truncated protein. He had no symptoms of spasticity at the time of his last clinical evaluation; however, the age at onset for HSP can vary from infancy to the 8th decade.[5] We cannot rule out that a genetic interaction is responsible for the severity of his

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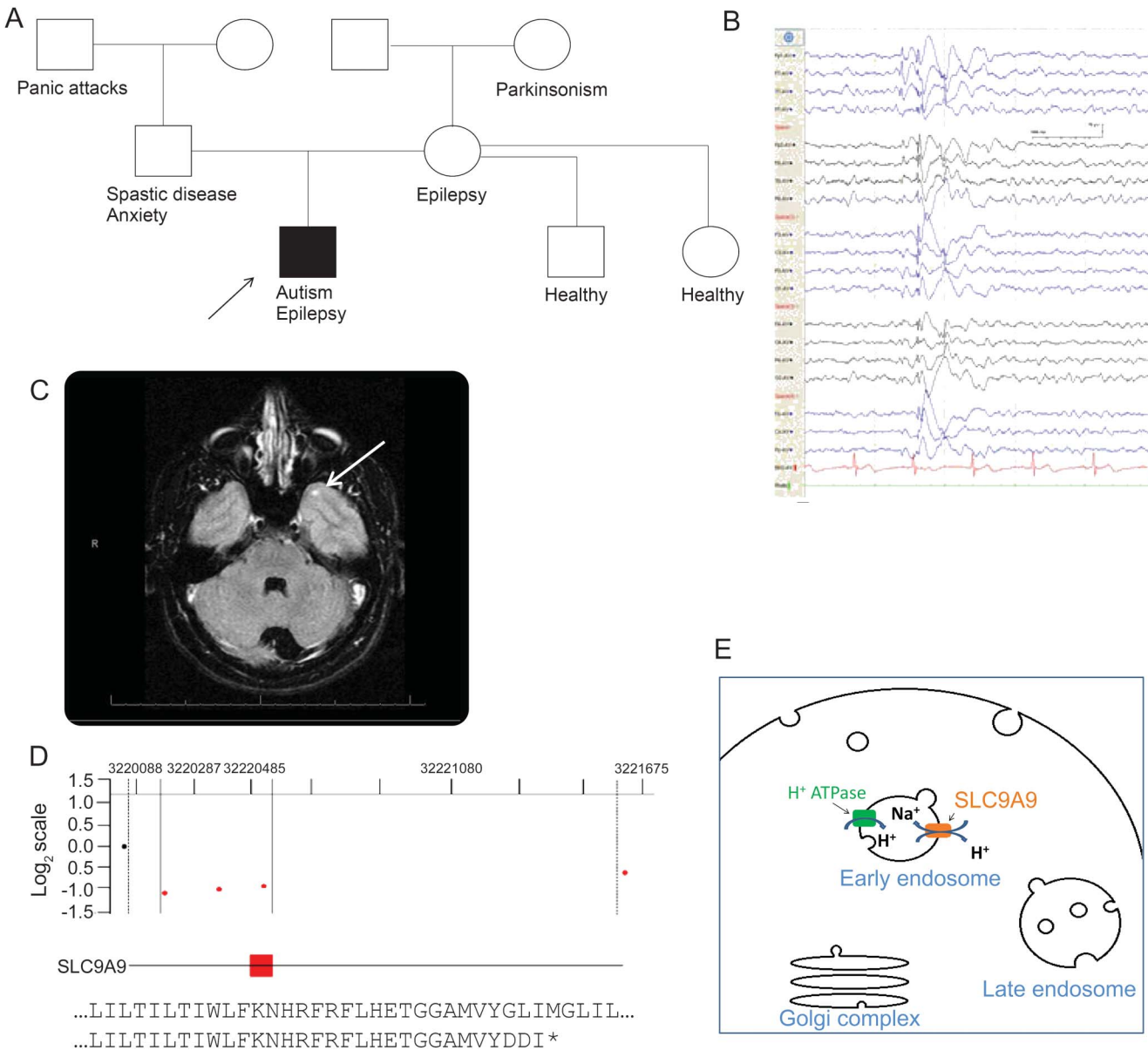
Figure **Clinical and molecular characterization**



(A) Pedigree of the individual with *SLC9A9* deletion. (B) EEG tracing revealing left frontotemporal focus of epileptiform activity. (C) T2-weighted MRI image with punctate temporal hyperintensity. (D) Array comparative genomic hybridization plot of region of chromosome 3q24 demonstrating exonic deletion of *SLC9A9* and predicted protein truncation. Y-scale is log2. Solid vertical lines represent minimally deleted regions (chr3:145033431-145033895), and dotted vertical lines represent maximally deleted region (chr3:145021878-145049622) based on hg18. Red circles are probes with a log2 ratio of −1.0 representing deletion of 1 copy in our patient, black circle with log2 of approximately 0. (E) Schematic representation of subcellular localization and function of NHE9.

phenotype, as has been identified in other individuals with developmental delay.[6]

Of note, there is an increased prevalence of epilepsy in individuals with autism. The prevalence of epilepsy in those with high-functioning autism is 9% and increases to 24% in those with comorbid intellectual disability.[7] The mechanism for this increased prevalence is currently unclear but may be due to similar molecular mechanisms, such as neuronal hyperexcitability. Together, our data and previously published reports suggest an important role for *SLC9A9* in a small subset of individuals with autism and epilepsy.

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