ARHGEF9 mutations cause a specific recognizable X-linked intellectual disability syndrome

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Mutations in more than 100 genes have been reported to cause X-linked intellectual disability (XLID), mainly in males. By contrast, the identified X-linked genes in which de novo mutations specifically cause ID in females are still limited so far.[1] Next-generation sequencing has nowadays enabled the screening for a causative mutation in XLID using targeted panel, X-exome, and whole-exome sequencing methods.[2] Mutations in the X-linked cell division cycle 42 guanine nucleotide exchange factor (GEF)-9 gene (*ARHGEF9*) have been recently associated with a wide phenotypic spectrum, including ID, behavior disorders, autism spectrum disorder, hyperekplexia, and infantile epileptic encephalopathy.[3–5] A number of patients with *ARHGEF9* mutations, encompassing missense and nonsense mutations, deletions, and complex rearrangements, have been reported. However, so far, the clinical features of *ARHGEF9* disease are nonspecific, with extreme phenotypic and genetic heterogeneity.

In this issue of *Neurology® Genetics*, Alber et al.[6] report 18 patients (including 5 females) identified through clinical practice (8 previously unpublished) and from a review of the literature. Detailed medical history and examination findings were obtained for each patient via a standardized questionnaire. Patients' age widely ranged from 4 to 57 years, and age at onset of the symptoms varied from day 1 to age 7 years, with a mean age of 15 months. Presenting symptoms included seizures (5 patients), developmental delay in combination with seizures (4 subjects), and developmental delay/ID alone (6 patients). Most patients (13 subjects) showed delayed early developmental milestones, and all individuals had ID ranging from mild-moderate to severe degree, with associated autistic features or hyperactivity/aggressive behavior in some cases. Thirteen patients had epilepsy with onset at a mean age of 20 months (range: 1 week–7 years). Seizure types were variable, including tonic-clonic seizures in 9 subjects, focal in 9, tonic in 2, and myoclonic in 1 individual. EEG recordings revealed generalized, focal, or even multifocal epileptic abnormalities. All patients with epilepsy were treated with antiseizure drugs, usually a polytherapy, and seizures were drug resistant in 4 cases. Minor abnormalities on neuroimaging were observed in 8 subjects and included nonspecific T2 hyperintensities/enlarged perivascular spaces in 3 subjects and hippocampal sclerosis, hypoplastic frontal lobe, brain atrophy, polymicrogyria, and delayed myelination in 1 each. Eight subjects had de novo hemizygous single nucleotide mutations, 3 had maternally inherited mutations, and 7 carried chromosomal disruptions. All female patients had strongly skewed X-inactivation. However, the most important merit of this article is to provide quite accurate genotype-phenotype correlations. Indeed, ID was moderate in individuals carrying with less severe mutations. Moreover, male patients were most severely affected with the overall neurologic syndrome (i.e., most severe ID and seizures) and showed distinctive and consistent facial features, including enlarged, fleshy ear lobes, and sunken appearance of the middle face (i.e., midface hypoplasia) in combination with prognathism. Finally, all the 3 male patients harboring mutations in exon 9 that do affect a specific pleckstrin homology (PH) domain of the protein did not develop epilepsy.

ARHGEF9 encodes collybistin, a protein which is highly expressed in multiple regions of the brain during development and structured by an N-terminal SRC homology 3 (SH3) domain followed by tandem DBL homology (DH), PH domains, and a C-terminal proline-rich sequence.[7] This protein structure identifies *ARHGEF9* as a member of the Rho GTPase activator family, responsible for the activation of Rho-family GTPases and involved in a number of cell signaling transduction pathways.[7] In addition,

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collybistin is essential for the clustering of the scaffolding protein gephyrin and of GABAA receptors at the postsynaptic membrane, 2 proteins associated with ID and epilepsy.[8] The reduction of inhibitory receptor clusters in the brain has been proposed as a plausible underlying pathophysiologic mechanism. In fact, collybistin-deficient male mice show reduced exploratory behavior, impaired spatial learning, increased anxiety scores, and reduction of gephyrin-dependent GABA receptor clusters in amygdala and hippocampus.[7]

The reasons behind the clinical variability of *ARHGEF9* mutations remain unknown and could be to be linked to several factors. First, the clinical features observed in mutated females may depend on the degree of X-inactivation skewing. Thus, while males have more pronounced ID and dysmorphic features, females may show variable symptoms with a phenotype similar to that of males when displaying the 100% X-inactivation.[9] However, X-inactivation and expression in blood might not necessarily reflect what is occurring in the brain, and other factors may modify expression, such as variants in regulatory sequences of *ARHGEF9* and related genes. Second, certain *ARHGEF9* mutations (e.g., C-terminal truncations) clearly cause dominant-negative effects on gephyrin and GABAA receptor clustering in neuronal systems.[10] Moreover, functional studies of *ARHGEF9* missense mutations affecting the PH domain structure that binds phosphatidylinositol-3-phosphate show consequent loss in the ability of collybistin to mediate gephyrin clustering.[10] Nevertheless, given the variability in clinical phenotypes associated with *ARHGEF9* mutations, it is evident that clinical observation, flanked by molecular genetics, is the best approach for a correct approach to patients with ID and other neuropsychiatric features. In fact, patients with ID often undergo a diagnostic odyssey before receiving the correct diagnosis, mainly because of clinical heterogeneity, unusual presentations, and lack of specific genotype-phenotype correlations. On the other hand, the identification of the disease-related mutation is of paramount importance to patients and their families, as awareness of the causing condition relieves uncertainty, improves communication with professionals, and provides prognostic information and enhances social support. Alber et al.[6] provide an excellent clinical genetic summary of the *ARHGEF9* mutations, providing great aid in the clinical dissection of *ARHGEF9* variants. Further observations and longitudinal follow-up studies are warranted in the future to clarify the full spectrum of *ARHGEF9* disease and to shed light on the accumulating evidence implicating neuronal synaptic gene products as key molecular factors underlying the etiologies of a diverse range of neurodevelopmental conditions.

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