NEW CANDIDATE GENES FOR INFANTILE SPASMS AND MENTAL RETARDATION

Disruption of the Serine/Threonine Kinase 9 Gene Causes Severe X-linked Infantile Spasms and Mental Retardation

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X-linked West syndrome, also called "X-linked infantile spasms" (ISSX), is characterized by early-onset generalized seizures, hypsarrhythmia, and mental retardation. Recently we showed that the majority of the X-linked families with infantile spasms carry mutations in the aristaless-related homeobox gene (*ARX*), which maps to the Xp21.3-p22.1 interval, and that the clinical picture in these patients can vary from mild mental retardation to severe ISSX with additional neurologic abnormalities. Here we report a study of two severely affected female patients with apparently de novo balanced X; autosome translocations, both disrupting the serine/threonine kinase 9 (*STK9*) gene, which maps distal to *ARX* in the Xp22.3 region. We show that *STK9* is subject to X-inactivation in normal female somatic cells and is functionally absent in the two patients, because of preferential inactivation of the normal X. Disruption of the same gene in two unrelated patients who have identical phenotypes (consisting of early-onset severe infantile spasms, profound global developmental arrest, hypsarrhythmia, and severe mental retardation) strongly suggests that lack of functional STK9 protein causes severe ISSX and that *STK9* is a second X-chromosomal locus for this disorder.

COMMENTARY

The molecular pathogenesis of infantile spasms (ISs) is an area of intense clinical and scientific investigation. Estimates are that ISs occur in two to five per 10,000 live births, and that mental retardation will develop in >70% of children with ISs. Whereas ISs may be seen in a variety of neurologic disorders, such as tuberous sclerosis, lissencephaly, or after hypoxic–ischemic injury, this epilepsy phenotype also may occur as a sporadic disorder. Recent evidence has shown that X-linked forms of ISs are associated with mutations in the *ARX* gene, which is a human homolog of the *Drosophila* aristaless gene. *ARX* is a homeobox-containing gene, and *ARX* mutations result in ISs, myoclonic seizures, and dystonia associated with mental retardation.

In this study, Kalscheuer and colleagues extend our vision of the molecular spectrum associated with X-linked forms of ISs by defining disruptions in the serine/threonine kinase 9 (*STK9*) gene in two unrelated female patients with ISs and mental retardation. Both patients exhibited X; autosome translocations and breakpoints at Xp22.3, a locus distal to *ARX*. Both patients also exhibited ISs and mental retardation clinically. Their electroencephalograms (EEGs) demonstrated hypsarrhythmia. No family history of consanguinity, epilepsy, or ISs was present. In one child (patient 1), magnetic resonance imaging (MRI) revealed ventricular enlargement as well as callosal and cerebellar hypoplasia. On physical examination, dysmorphic facial features, including hypertelorism, high nasal bridge, and a simian crease were found. In the second patient (patient 2), the MRI was normal, and facial morphology was normal. Patient 1 exhibited a balanced translocation between the distal short arm of chromosome X and the short arm of chromosome 7, whereas patient 2 was found to have a translocation between the distal short arm of chromosome X and the proximal short arm of chromosome 6. Because neither translocation was detected in the parents of these children, they were viewed as de novo translocations. The authors performed experiments to demonstrate 100% inactivation of the normal X-alleles in these two females, resulting in full expression of the mutant-containing allele. Subsequent sequencing of the region of *STK9* revealed a breakpoint within intron 10 in patient 1, whereas in patient 2, the break was mapped to a region within 2 kilobases of *STK9* intron 1. Reverse transcriptase–polymerase chain reaction (RT-PCR) amplification of *STK9* in patient 1, with breakpoint-flanking primers in exons 9 and 12, did not yield a product nor did primers that were 3′ to the breakpoint. These results suggested that in this case, the intron 10 break led to a functional deletion

of the remaining 11 exons (*STK9* contains ≥23 exons) and truncation of the STK9 protein. In patient 2, no *STK9* mRNA could be detected by RT-PCR. Sequence and expression of *ARX* in these two patients was normal. Finally, screening of the four published families with known mutations in the *ARX* gene did not reveal *STK9* mutations.

These results demonstrate that the molecular spectrum of events leading to ISs is very likely broad and quite heterogeneous. The appearance of a balanced translocation affecting *STK9* provides a new candidate gene to account for ISs that is distinct from *ARX*. At the functional level, these two genes also are quite distinct (a putative transcription factor and a serine/threonine kinase), suggesting that divergent mechanisms may lead to the common clinical phenotype of ISs. The function of the STK9 protein remains to be more fully defined. STK9 is a member of the larger family of serine/threonine protein kinases that are ubiquitously expressed. Structural homologs of STK9, including KKIAMRE, are members of the mitogen-activated protein (MAP) kinase family that may be pivotal in synaptic plasticity, cell division, and neuronal survival. A logical extension of these studies will be to investigate whether spontaneous mutations in *STK9* or *ARX* can be identified in sporadic ISs (West syndrome) patients. If so, these candidate genes would provide invaluable insights into potential developmental abnormalities that lead to ISs and, perhaps, even highlight new target molecules for therapeutic development.

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