Westward Ho! Pioneering Mouse Models for X-Linked Infantile Spasms Syndrome

Targeted Loss of Arx Results in a Developmental Epilepsy Mouse Model and Recapitulates the Human Phenotype in Heterozygous Females. Marsh E, Fulp C, Gomez E, Nasrallah I, Minarcik J, Sudi J, Christian SL, Mancini G, Labosky P, Dobyns W, Brooks-Kayal A, Golden JA. *Brain* 2009;132(Pt 6):1563–1576. Mutations in the X-linked aristaless-related homeobox gene (ARX) have been linked to structural brain anomalies as well as multiple neurocognitive deficits. The generation of Arx-deficient mice revealed several morphological anomalies, resembling those observed in patients and an interneuron migration defect but perinatal lethality precluded analyses of later phenotypes. Interestingly, many of the neurological phenotypes observed in patients with various *ARX* mutations can be attributed, in part, to interneuron dysfunction. To directly test this possibility, mice carrying a floxed *Arx* allele were generated and crossed to *Dlx5/6*$^{CRE\text{-}IRES\text{-}GFP}$(*Dlx5/6*^{CIG}) mice, conditionally deleting *Arx* from ganglionic eminence derived neurons including cortical interneurons. We now report that *Arx*$^{-/y}$;*Dlx5/6*^{CIG} (male) mice exhibit a variety of seizure types beginning in early-life, including seizures that behaviourally and electroencephalographically resembles infantile spasms, and show evolution through development. Thus, this represents a new genetic model of a malignant form of paediatric epilepsy, with some characteristics resembling infantile spasms, caused by mutations in a known infantile spasms gene. Unexpectedly, approximately half of the female mice carrying a single mutant *Arx* allele (*Arx*$^{-/+}$;*Dlx5/6*^{CIG}) also developed seizures. We also found that a subset of human female carriers have seizures and neurocognitive deficits. In summary, we have identified a previously unrecognized patient population with neurological deficits attributed to ARX mutations that are recapitulated in our mouse model. Furthermore, we show that perturbation of interneuron subpopulations is an important mechanism underling the pathogenesis of developmental epilepsy in both hemizygous males and carrier females. Given the frequency of *ARX* mutations in patients with infantile spasms and related disorders, our data unveil a new model for further understanding the pathogenesis of these disorders.

A Triplet Repeat Expansion Genetic Mouse Model of Infantile Spasms Syndrome, *Arx*$^{(GCG)10+7}$, with Interneuronopathy, Spasms in Infancy, Persistent Seizures, and Adult Cognitive and Behavioral Impairment. Price MG, Yoo JW, Burgess DL, Deng F, Hrachovy RA, Frost JD Jr, Noebels JL. *J Neurosci* 2009;29(27):8752–8763. Infantile spasms syndrome (ISS) is a catastrophic pediatric epilepsy with motor spasms, persistent seizures, mental retardation, and in some cases, autism. One of its monogenic causes is an insertion mutation [c.304ins $(GCG)_7$] on the X chromosome, expanding the first polyalanine tract of the interneuron-specific transcription factor *Aristaless*-related homeobox (ARX) from 16 to 23 alanine codons. Null mutation of the *Arx* gene impairs GABA and cholinergic interneuronal migration but results in a neonatal lethal phenotype. We developed the first viable genetic mouse model of ISS that spontaneously recapitulates salient phenotypic features of the human triplet repeat expansion mutation. *Arx*$^{(GCG)10+7}$ ("*Arx* plus 7") pups display abnormal spasm-like myoclonus and other key EEG features, including multifocal spikes, electrodecremental episodes, and spontaneous seizures persisting into maturity. The neurobehavioral profile of *Arx* mutants was remarkable for lowered anxiety, impaired associative learning, and abnormal social interaction. Laminar decreases of Arx+ cortical interneurons and a selective reduction of calbindin-, but not parvalbumin- or calretinin-expressing interneurons in neocortical layers and hippocampus indicate that specific classes of synaptic inhibition are missing from the adult forebrain, providing a basis for the seizures and cognitive disorder. A significant reduction of calbindin-, NPY (neuropeptide Y)-expressing, and cholinergic interneurons in the mutant striatum suggest that dysinhibition within this network may contribute to the dyskinetic motor spasms. This mouse model narrows the range of critical pathogenic elements within brain inhibitory networks essential to recreate this complex neurodevelopmental syndrome.

COMMENTARY

William James West described the syndrome that now bears his name after observing the devastating consequences of infantile spasms in his own child (1). The diagnosis of West syndrome, including the subgroup with X-linked infantile spasms, is typically made in the first year of life, when affected children develop spasms or myoclonic jerks that resemble an exaggerated Moro reflex. Many patients have a signature interictal EEG that is "a picture of electroencephalographic chaos," with high-voltage, disorganized waves and multifocal sharp spikes (2). The classical West syndrome EEG, shown in Figure 1, is called hypsarrhythmia (2). The prognosis is poor, even when the EEG is normalized by treatment with adrenocorticotrophic hormone or drugs, such as vigabatrin. Though the hypsarrhythmia subsides in nearly one-quarter of the patients, most have epileptic seizures, progressive cognitive decline, dystonia, spasticity, and severe mental retardation (1).

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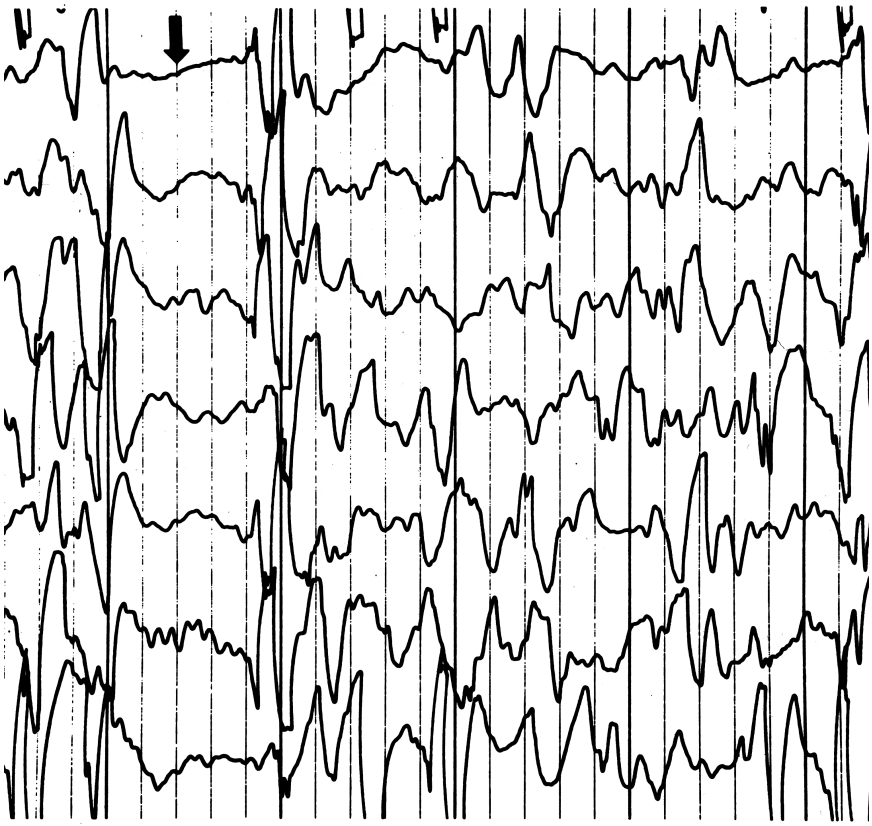

FIGURE 1. EEG of a child with West syndrome/infantile spasms. Infantile spasms are coupled to a characteristic age-dependent EEG pattern called hypsarrythmia. The pattern consists of chaotic spikes, polyspikes and slow waves, interspersed with burst-suppression periods (see arrow). (Reproduced with permission from Freeman JM. In: Moshe SL, Schwartzkroin PA, Noebels JL, Swann JW, eds. *Brain Development and Epilepsy*. New York: Oxford University Press; 1995, pp. 9–33.)

While the basis for most infantile spasms is unknown, findings in worms, mice, and humans suggest that it can be caused by genetic mutations that result from defects in neurotransmitter release (3–5) or impair neuronal migration (6). More than 60 distinct disease-associated mutations in seven X-linked mental retardation syndromes are known. One of these mutations occurs in the Aristaless-related homeobox gene (ARX) that maps within a locus on Xp22 (7,8). Seizure disorders associated with *ARX* mutations include West syndrome, X-linked myoclonic epilepsy, Partington syndrome, and nonsyndromic X-linked mental retardation (8,9). Infantile spasms can also result from infections, head trauma, or hypoxic–ischemic injury. The heterogeneous etiologies of infantile spasms point to links between pediatric seizures and disruptions in developmental events or neural pathways. To study the mechanisms by which the underlying lesion, brief seizures, or hypsarrhythmia disrupt developmental processes and lead to permanent cognitive deficits, improved animal models of West syndrome are needed (10). Now there are two mouse genetic models for West syndrome that show hypsarrhythmia, profound reductions in forebrain GABAergic interneurons in the adult brain, and concomitant cognitive deficits.

ARX belongs to a family of homeodomain-containing transcription factors that enhance or repress different genes in various tissues. Conserved domains, such as the *aristaless* domain and the *prd*-like class homeodomain, regulate the expression of many genes involved in embryo patterning, possibly explaining why infants with mutations in these homeodomains show catastrophic brain malformations (11). A third domain contains repeating polyA tracts (translated into alanines). Common mutations increase the polyA tracts and with them, the repression of target genes (11). Four polyA tracts are in the gene, including a mutation-prone hot spot in the second exon. The length of the polyA tracts couples with the severity of the disorder: shorter expansions of one to three alanines cause mental retardation (12), while longer expansions, which create seven extra alanine residues, cause infantile spasms and West syndrome (13). The longest mutation expands to 27 alanine residues and causes early infantile epileptic encephalopathy with a suppression burst pattern (14).

Postmortem analyses of a patient with an *ARX* mutation showed that abnormally few GABAergic neurons are located in the cerebral cortex, but many appear in the white matter and subventricular zones—regions that do not normally contain interneurons (6). Kato and Dobyns proposed that deficiencies in GABAergic interneurons might cause infantile spasms, possibly by deregulating a common ontological pathway for interneuron development (15). Their hypothesis, termed interneuronopathy, attempts to unify the clinical symptoms with neuropathological abnormalities. Until recently, scientists lacked

the ability to directly test the interneuronopathy hypothesis because *Arx* null mice, which also have disturbances in forebrain interneuron distributions, die at birth (7,16). Thus, the *Arx* null mice do not live long enough to examine their EEG patterns or behavior, making it impossible to verify if the deficits in interneuron positioning cause motor spasms and hypsarrhythmia.

Currently, two independent groups, reviewed here, have created nonlethal *Arx* deficits in mice and have demonstrated how *ARX* mutations cause dysfunctional interneurons. Marsh et al. created an allele of *Arx* flanked by *lox-p* sites. The usefulness of this construct is that any allele flanked by these sites (i.e., floxed alleles) is excised in the presence of the enzyme Cre-recombinase. The floxed mice were mated to a mouse that expresses Cre-recombinase under the control of the *Dlx5/6* enhancer element which is active in calbindin interneuron subtypes. Neither transgene alone causes a defect, however, when the two strains are mated, the male offspring become *Arx* deficient ($Arx^{-/}$ *Y;Dlx5/6* ^{CIG}) and the female offspring have only one functional copy of *Arx* ($Arx^{-/+}$*;Dlx5/6* ^{CIG}).

EEG abnormalities were found in the *Arx*-deficient mice on postnatal days 14–17. All of the $Arx^{-/}$*Y;Dlx5/6* ^{CIG} males, and a majority of the *Arx*-deficient female mice showed spontaneous seizures. EEG abnormalities were further evaluated with continuous video and intracranial EEG recordings for up to 1 month when the mice were 3- to 4-months old. The male *Arx*-deficient mice also showed reduced theta and delta activity. Deficits in various GABAergic interneuron populations in these mice correlate with the EEG findings.

A second study by Price et al. examined the effects of *Arx* mutations consisting of the triplet repeat expansion, which is found in multiple human patients (13). They created a transgene with repeating stretches encoding for the amino acid, alanine, in critical exons of the *Arx* gene. In exon 2 of the human *ARX* gene, the polyA tract is normally 10 codons long, but an insertion of seven repeats of the trinucleotide sequence, guanine–cytosine–guanine (this DNA trinucleotide is the codon for the amino acid alanine), occurs in West syndrome. The result is a longer tract of 23 alanines in the protein. This mutation is designated *ARX*$^{(GCG)10+7}$. Price et al. created a similar insertion mutation by targeting a construct of the mouse *Arx* gene that causes expansion of the initial polyA tract in exon 2. They designated this mouse line $Arx^{(GCG)10+7}$.

Postnatal $Arx^{(GCG)10+7}$ mice showed infantile motor spasms, spontaneous seizures, and EEG abnormalities, including sharp-spike, slow-wave transients; electrodecremental EEG events; and increased high-frequency background rhythmic activity. They also demonstrated cognitive impairments. In addition, the mutants displayed reduced anxiety-like behaviors on a light/dark exploration test, compared to wild-type mice. When tested in a pavlovian associative learning paradigm that pairs a tone with a brief foot shock, the mutant mice show a deficit in learning and memory. A final test described a measure of social interactions with other mice. When confronted with an unfamiliar mouse in their home cage, most mice actively investigate the interloper. However, the *Arx* mutant mice retreat, an anomalous social behavior.

Immunohistochemical studies evaluating the distribution of ARX-expressing GABAergic interneurons showed that they were almost entirely missing in the cerebral cortex, the hilus of the dentate gyrus, and the striatum of the mutant mice. Surprisingly, interneurons show normal distributions in the parietal cortex, whereas the overall decrement in GABAergic cortical interneurons in the $Arx^{(GCG)10+7}$ mice is approximately 50%. Moreover, the mice have fewer calbindin$^+$ and neuropeptide Y$^+$ interneurons, but normal distributions of parvalbumin$^+$ and calretinin$^+$ interneurons. Though it seems likely that reduced inhibitory neuron numbers in the cerebral cortex and hippocampus account for the clinical EEG features, hypsarrhythmia also could be related to a region and area-specific cortical circuit anomaly. With these new mouse models, it may now be possible to map the functional neural circuits underlying age-related EEG anomalies.

Of what consequence are the trinucleotide expansions for the transcriptional activity of ARX protein in the immature brain? ARX normally localizes to the nucleus, but it becomes cytoplasmic in the mutant mice (17). Expansion mutations in other transcription factor genes can cause proteins to misfold, resist degradation, and form aggregates in the cytoplasm. Such changes could cause subtle impairments in transcriptional regulation and neuronal development that may have profound clinical implications, as the expression of over 84 downstream target genes is altered by disrupting *Arx* (18). Now the search is on for interneuron-specific genes responsible for the development of interneuronopathy and for how such gene expression is changed in *ARX* mutations (19). The pioneering work presented in these two papers allows researchers to embark on an extremely promising adventure across uncharted territory in the molecular biology of West syndrome.

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