Short Report

Early-onset epileptic encephalopathy in a girl carrying a truncating mutation of the ARX gene: rethinking the ARX phenotype in females


Severe early-onset epilepsy is due to a number of known causes, although a clear etiology is not identifiable in up to a third of all the cases. Pathogenic sequence variations in the ARX gene have been described almost exclusively in males, whereas heterozygous female relatives, such as mothers, sisters and even grandmothers have been largely reported as asymptomatic or mildly affected. To investigate the pathogenic role of ARX in refractory epilepsy of early onset even in females, we have screened the ARX sequence in a population of 50 female subjects affected with unexplained epileptic encephalopathy with onset in the first year of life. We report the identification of a novel truncating mutation of the coding region of the ARX gene in a girl with a structurally normal brain. Our findings confirm the role of ARX in the pathogenesis of early epilepsy and underline the importance of screening of the ARX gene in both male and female subjects with otherwise unexplained early onset epileptic encephalopathy.

Conflict of interest

The authors declare no conflicts of interest.

Severe early onset epilepsy is a devastating condition because of a number of known causes, although a clear etiology is not identifiable in up to a third of all the cases.

The aristaless-related homeobox gene ARX (OMIM *300382) is a developmentally regulated homeobox transcription factor with a fundamental role for normal brain development. It is expressed in the developing hypothalamus, thalamus, basal ganglia and cerebral cortex, modulates migration and fate specification of interneurons, and regulates ventricular zone proliferation (1).

ARX is a major player in the etiopathogenesis of refractory early onset epilepsy with severe and global neurological impairment with or without brain malformations. On the other hand, pathogenic sequence variations in the ARX gene have been identified in a broad range of phenotypes, making it one of the most pleiotropic disease-causing genes. Reported affected individuals are predominantly males; the female phenotype, almost exclusively defined through the identification of mothers or other female relatives of male probands, includes alterations of the corpus callosum, and sometimes mild intellectual disability possibly associated with epilepsy and/or psychiatric phenomena including anxiety, depression, and schizophrenia (2).

In order to investigate the pathogenic role of ARX in refractory epilepsy of early onset in females, we have
screened a population of 50 female subjects affected with unexplained epileptic encephalopathy with onset in the first year of life.

The identification of a de novo deleterious mutation in the coding region of the ARX gene in one of the affected girls stresses the importance of considering ARX in the genetic workup of otherwise unexplained early-onset epileptic encephalopathy, both in male and female subjects whether they be familial or sporadic cases.

Methods

Patient recruitment

Female subjects with a diagnosis of epileptic encephalopathy and onset of seizures in the first year of life, including sporadic and familial cases, were recruited for the study.

Key clinical inclusion criteria were: absence of a recognizable cause for epilepsy, based on anamnesis, clinical evaluation, neurophysiological and neuroradiological assessments and negative standard cytogenetic analysis. A comprehensive set of clinical data including seizure history, electroencephalographic recordings and magnetic resonance imaging (MRI) findings, was evaluated.

Genetic analysis

Genomic DNA was extracted from peripheral blood leukocytes (PBL) with standard methods. The coding region of the ARX gene was amplified by polymerase chain reaction as previously described (3) and analyzed by denaturing high-performance liquid chromatography (dHPLC) with WAVE® MD Nucleic Acid Fragment Analysis System (Transgenomic, Inc., Omaha, NE). PCR products with abnormal dHPLC profiles were sequenced on ABI 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA). Gene dosage analysis was performed by real-time quantitative PCR of exonic fragments on ABI PRISM® 7000 Sequence Detection System (Applied Biosystems, Foster City, CA).

X-chromosome inactivation

A methylation-specific PCR at the Human Androgen Receptor gene (HUMARA) polymorphism was performed using a standard method (4). The degree of skewing of X-inactivation was calculated as the fractional peak height ratio (expressed as a percentage) for the more strongly amplified allele. X-inactivation was considered significantly skewed at a ratio of 75:25 or higher. DNA samples from the parents were genotyped to determine the parental origin of alleles.

Results

Population and molecular data

Fifty female individuals, with age ranging between 1 month and 16 years, were recruited for the study based on the above mentioned inclusion clinical criteria. These cases, all sporadic, were negative for a previous mutation scanning (sequence variants and deletions/insertion events) of the CDKL5 gene.

The molecular analysis allowed the identification of a pathogenic ARX sequence variant in one of the 50 girls tested (2%). The alteration, a frameshift mutation in exon 5 (c.1459delA), was predicted to yield a protein prematurely truncated at position 491 (p.Thr487GlnfsX5) (Fig. 1a,b). The mutation, not detected in at least 100 X chromosomes from normal individuals, was not present in mutation databases and was not previously reported in literature. Moreover, it was not detected in the DNA of either parent and was therefore considered a de novo mutation.

The X-inactivation analysis at the androgen receptor locus showed a ratio of 70:30 consistent with a partially skewed X-inactivation, with the paternal allele as the prevalently active one.

Case description

The patient we describe is the third child of healthy non-consanguineous parents. The two older siblings were healthy boys (Fig. 2a). The girl was born at term after an uneventful pregnancy and delivery, and had an unremarkable perinatal period. Her birth weight, length and OFC were reportedly normal. She was first brought to medical attention at 3 months of age because of the onset of multiple daily brief attacks of hypertonic hyperextension of the four limbs with conjugate deviation of the eyes to the right and perioral cyanosis followed by clusters of flexor spasms, nystagmus and loss of consciousness. At that time, the neurological examination showed global psychomotor delay with poor interaction, absent social smile and no reactivity to acoustic and visual stimuli. A divergent strabismus was evident. The child had no head control; deep tendon reflexes were brisk with a positive Babinski sign bilaterally as well as bilateral ankle clonus. The fundus oculi examination was normal. She had normal somatic growth without clinical evidence of congenital malformations or significant dysmorphic features (Fig. 2b). A congenital hip dislocation was clinically suspected and confirmed by ultrasound scan. An electroencephalogram (EEG) was performed which showed normal background activity both when awake and during sleep. The sleep recording displayed recurrent high-voltage bi-phasic, synchronous and asynchronous, central and post-central spikes, predominantly in the right hemisphere.

Treatment with vigabatrin and nitrazepam was initially started with poor results, and only treatment with adrenocorticotropic hormone (ACTH) led to cessation of seizures from the age of 9 months.

The psychomotor development was always very slow; she gained head control at 13 months and could sit unsupported at 18 months. She never attained urinary or fecal continence. Around the age of 18 months repetitive stereotypic hand movements became apparent, reminiscent of hand-washing or hand-wringing, as
well as increasing deviation of gaze. She continued to have little interest in people and objects. Autistic features with severely impaired communication and reciprocal social interaction became prominent from the age of 2 years.

When last reviewed, at the age of 3 years, her language skills were severely impaired with absence of speech; she could walk with unilateral support, although she was significantly ataxic, and showed very poor gross and fine motor skills.

During these early years several investigations were carried out. Two brain MRI scans of the brain performed at the age of 3 months and 2 years were unremarkable (data not shown). In particular, no cortical malformations, atrophy or hypoplasia of cerebellum or corpus callosum, were evidenced. Neurophysiologic investigations [visual evoked potentials (VEP), auditory brainstem response (ABR), somatosensory evoked potential (SSEP), electromyography (EMG) and neurography] were all normal.

Routine laboratory work-up and metabolic tests (including plasma lactate and pyruvate, ammonia, urine organic acids, plasma acylcarnitine, and plasma aminoacids) were repeatedly normal. A standard cytogenetic analysis revealed a normal 46, XX karyotype. CDKL5 molecular testing did not show any alterations of the coding sequence of the gene.

Discussion

Pathogenic sequence variations in the ARX gene have been implicated in several different phenotypes, which underlies its pleiotropic effect in human diseases (5, 6). The ARX protein contains important functional domains such as the octapeptide (OP, aa 27–34), three nuclear localization signals (NLS, aa 82–89, 325–332, and 379–386), four polyalanine tracts (polyA, aa 100–115, 144–155, 275–281, and 432–440), the paired-like class homeodomain (Prd, aa 328–387) and the Aristaless domain (OAR, aa 527–542) (7). Different types of mutations of the ARX gene have been described: the majority of the mutations, so far reported, consist of expansions of the polyalanine tracts, with the second tract being the most frequently involved one. Genotype–phenotype correlations appear to suggest that polyalanine tract expansions are more frequently associated with a non-malformative phenotype, whereas large intragenic deletions frameshifts or nonsense mutations are more typically identified in subjects with brain and/or genital malformations (5, 8).
Neurological conditions associated with ARX mutations have so far been almost exclusively reported in male individuals, whereas the female phenotype, largely ascertained through the identification of mothers or other relatives of male probands, has included alterations of the corpus callosum, sometimes with mild intellectual disability, possibly associated with epilepsy (9) and/or with anxiety, depression, and schizophrenia (2).

Very few of the females reported as affected had epilepsy with onset in the first year of life (5, 9–11) while, to the best of our knowledge, in only one case a non-malformative early onset epileptic encephalopathy was identified (10).

We describe here the case of a girl, born after an unremarkable pregnancy, with a normal perinatal period and with no relevant family history, who presented at the age of 3 months with intractable seizures configuring an electroencephalographic picture of early onset epileptic encephalopathy. Two MRI scans of the brain performed before 3 years of age showed no structural abnormalities.

Molecular analysis of the ARX gene led to the identification of a frameshift mutation predicted to result in a prematurely truncated protein or possibly an absent protein product as a consequence of nonsense mediated decay of the ARX transcript. This novel ARX mutation was not present on the DNA of either parent of the proband.

Interestingly, the only other female with non-malformative early onset epileptic encephalopathy associated with an ARX alteration reported in literature was found to carry a different frameshift mutation (c.1465delG, p.A488fs) (10) that was co-incidently predicted to generate a stop codon at position 491 of the ARX gene, exactly the same amino acid residue as the mutation found in the girl we report.

These two truncating mutations lead to a loss of the extremely conserved Aristaless domain region of the protein suggesting that if the truncated protein is made, key protein–protein interactions would be disrupted. The de novo nature, together with the molecular characteristics of the sequence variant we report, are strong indicators of its pathogenicity and therefore of its causal involvement in the patient’s phenotype. On the other hand, the skewed pattern of X-chromosome inactivation found in this girl is with high likelihood another important factor of the final phenotypic expression in the patient. In both cases with predicted truncation of the ARX protein at nucleotide 491, a skewed XCI pattern has been detected that can be interpreted as an unfavorable epigenetic modulator of the phenotype.

The case we report underscores some important conclusions: (i) the absence of cortical malformations further substantiates the hypothesis that ARX mutations themselves can generate a severe and epileptogenic disorganization of neuronal networks probably because of GABAAergic interneuron dysfunction, permitting the classification of these clinical conditions as interneuronopathies (12, 13), (ii) truncating mutations of the ARX gene, and not only expansions of the polyalanine tracts, can result in a non-malformative phenotype, (iii) a pathogenic mutation of the ARX gene can, possibly in association with a skewing of the X-chromosome inactivation, be responsible of epileptic encephalopathy or early and severe neurological manifestations, even in females.

This report further confirms the pathogenic role of the ARX gene in early onset epileptic encephalopathies and strongly suggests that ARX molecular analysis should be extended to female patients.

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Ethics approval

Regular informed consent for genetic testing was obtained from the parents of all the children included in this study, according to the requirements of the local ethical review board.

References