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Case Report

ARX-associated infantile epileptic-dyskinetic encephalopathy with responsiveness to valproate for controlling seizures and reduced activity of muscle mitochondrial complex IV

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Abstract

Background: ARX genetic defect is associated with a spectrum of neurodevelopmental disorders that exhibit a high degree of phenotypic heterogeneity.

Methods: We studied a family with a 13-year old Chinese boy and his two elder brothers presented with infantile epileptic-dyskinetic encephalopathy and clarified the unknown genetic etiology of the youngest brother by whole exome sequencing.

Results: The youngest brother of this family presented with developmental regression, dystonia, epilepsy, microcephaly, visual impairment and oromotor dysfunction. Hyperlactataemia, raised alanine and muscle complex IV deficiency indicated that he had mitochondrial dysfunction. Likely pathogenic hemizygous missense *ARX* variants (c.989G > A; p.Arg330His) located in conserved nuclear localization sequence was identified. The variant was carried by his asymptomatic mother and not found in his asymptomatic third elder brother. The intractable seizures showed complete but transient responsiveness to pyridoxal phosphate and finally controlled by valproate treatment.

Conclusion: This is the first case of *ARX*-associated encephalopathy showing mitochondrial dysfunction and transient responsiveness to pyridoxal phosphate treatment.

Keywords: *ARX*; Aristaless-related homeobox; Infantile epileptic-dyskinetic encephalopathy; Mitochondrial dysfunction; Pyridoxal phosphate

1. Introduction

ARX is the Aristaless-related homeobox gene inherited in an X-linked recessive manner. It encodes a transcription factor that acts as both transcriptional activator and repressor in various fundamental brain developmental processes [1]. It consists of a paired-like homeodomain, a C-terminal Aristaless domain, an octapeptide domain located near N-terminus, three nuclear localization sequences (NLS), four polyalanine tracts and a central acidic domain [1]. Previous reports indicated that disorders of *ARX* genetic defects exhibited a high degree of phenotypic heterogeneity and were

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associated with a spectrum of neurodevelopmental disorders ranging from severe brain structural anomalies to a non-malformation group [2]. No mitochondrial dysfunction has been reported in *ARX*-associated syndromes.

We studied a family with five offspring in which three affected ones presented with infantile epileptic-dyskinetic encephalopathy. The clinical phenotype and the biochemical parameters, including oxidative phosphorylation enzymes in skeletal muscle, of the youngest boy were compatible with a mitochondrial disorder (MD). Subsequent molecular analysis revealed a likely pathogenic *ARX* variant. The intractable seizures responded only transiently to pyridoxal phosphate (PLP) treatment.

2. Case report

This 13-year old Chinese boy is the fifth offspring of a healthy Chinese non-consanguineous couple. Since early infancy, he had severe global developmental delay with the overall mental age of less than 3 months all along, bilateral cortical visual impairment and progressive dystonia with an episode of status dystonicus and rhabdomyolysis without obvious precipitating factors. The dystonia was controlled with benzhexol and baclofen. He also suffered from intractable infantile-onset epileptic spasms, focal seizures and generalized myoclonic seizures. The focal seizure involved left facial twitching, versive head and eyes movements to the left side followed by generalized (a)symmetrical tonic stiffening of the whole body. However, the family initially defaulted all neurology follow up in another hospital until nearly 3 years of age when he presented to our centre, conventional treatment for infantile spasms such as steroid and vigabatrin was not given. His epilepsy failed to respond to various anticonvulsants including phenobarbital, clobazam, lamotrigine, levetiracetam, nitrazepam and ketogenic diet. The patient continued to have daily cluster of epileptic spasms with 5 to 10 prolonged 10-minute focal seizures. Over the years, he developed progressive microcephaly, poor weight gain and oromotor dysfunction requiring gastrostomy feeding solely. Physical examination, other than dystonia and short statue, was unremarkable. No clinical myopathy, hypertrichosis nor definite fatiguability were noted. Serial electroencephalographies (EEG) revealed slow background for age, abnormal sleep changes with generalized and focal left occipital discharges. Magnetic resonance imaging of the brain at 3 years old showed squaring of anterior horn of lateral ventricles with no other abnormalities. Currently, he has profound intellectual disability and is wheelchair-bound due to generalized dystonia.

Biochemical investigations revealed lactic acidaemia (2.4–3.3; normal <2 mmol/L) with a raised lactate/pyruvate ratio to 31 (normal <=18) and hyperalaninaemia (815–946; normal 150–545 μmol/L). Urine organic acid analysis showed no abnormalities. Lactate from the cerebrospinal fluid was normal. Magnetic resonance spectroscopy was not performed. Muscle histology did not reveal any abnormality including COX-deficient fibres and electron microscopy study was normal. Respiratory chain analysis of muscle revealed a reduced complex IV activity of 350 mU/UCS (control range: 520–2080 mU/UCS) while the activities of complex I and citrate synthase were within normal range. There was insufficient tissue available for measurement of complexes II and III. Based on all findings, our patient was highly suspected to have a MD, with a score of 8 i.e. definite MD, under the Mitochondrial Disease Criteria [3]. Whole mitochondrial DNA (mtDNA) sequencing in the muscle identified no pathogenic variant.

The eldest and the second eldest brother of this patient suffered from a similar phenotype with early-onset epilepsy and severe global developmental delay. Details of the clinical histories were unknown as both of them did not have active follow up in our centre. The two parents, his elder sister and the third eldest brother were healthy.

A hemizygous missense *ARX* variant (NM_139058.3: c.989G > A; p.Arg330His) was identified in our 13-year old index patient by whole exome sequencing, bioinformatics analysis and subsequent Sanger sequencing confirmation (Fig. 1). This substitution is located in the conserved N-terminal NLS2 flanking ARX homeodomain. The amino acid residue Arg330 mutated in the patient is highly conserved by ClustalW multiple sequence alignment of ARX in different species. In silico analysis including SIFT, Polyphen-2 and Mutation Taster predicted that this variant is damaging to the protein structure and function. This variant was not found in the normal individuals including East Asian ancestry in The Genome Aggregation Database (gnomAD). Using the 3D modeling by STRUM, the p.(Arg330His) variant results in a delta-delta G value of −1.44. A negative delta-delta G value implies the variant caused fold destabilization of the ARX protein [4].

Segregation analysis showed that his asymptomatic mother and elder sister were carriers of the p.Arg330His variant. The variant was not found in his asymptomatic father and asymptomatic third elder brother (Fig. 1). Unfortunately, we did not have the DNA of the other 2 affected siblings. According to ACMG standards and guidelines, this variant is classified as "likely pathogenic". The variant is also classified as "likely pathogenic" in ClinVar of NCBI. No pathogenic variants were found in gene panels associated with MDs and with strong support of mitochondrial localization suggested in MitoCarta 2.0.

As he suffered from infantile onset epileptic encephalopathy, PLP treatment at a dose of 200 mg

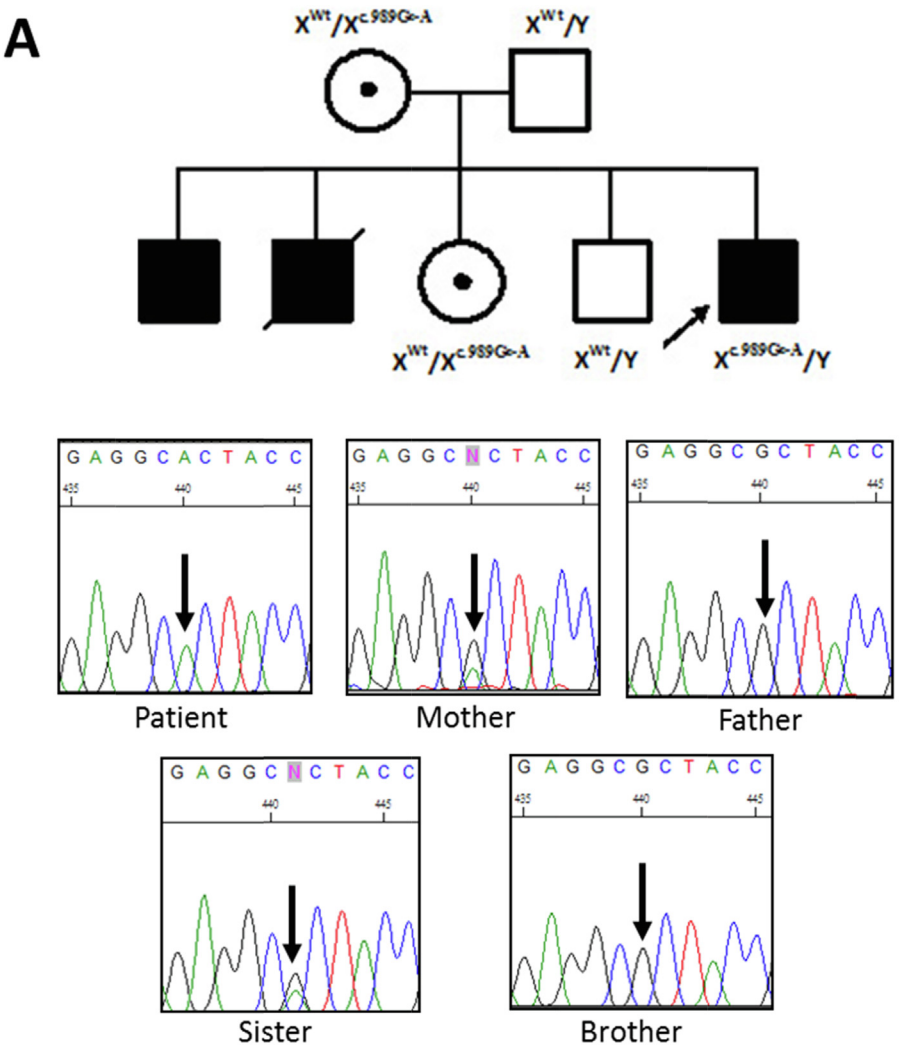

Fig. 1. A. Pedigree of the family with ARX mutations and Sanger sequencing results. A hemizygous missense ARX variant (c.989G > A; p. Arg330His) was identified in our 13-year old index patient indicated by an arrow and it is not found in his father and brother. Mother and sister are the carriers of the variant. B. Multiple sequence alignment of different species. Amino acid residue Arg330 is highly conserved among different species.

every 4 h (44 mg/kg/day) was tried before his molecular diagnosis. Surprisingly, the seizures stopped completely for six months before recurrence again and weaning of PLP caused aggravation of seizures, making him PLP-dependent. No EEG was performed immediately before and after PLP treatment. Unfortunately, we did not

have access to analyse the level of PLP in the cerebrospinal fluid. After identification of the *ARX* variant, primary MD was excluded and valproate treatment was commenced after the patient failed PLP trial and his seizure was subsequently under control. Serial monitoring did not show deterioration of biochemical markers of mitochondrial dysfunction such as lactate and alanine.

3. Discussion

The hemizygous variant (c.989G > A; p.Arg330His) identified in the present study was located in the conserved NLS2 region flanking ARX homeodomain. Previous studies identified several point mutations in this region with p.Arg332Pro, p.Arg332His and p.Arg332Cys resulting in X-linked lissencephaly with abnormal genitalia and p.Thr333Asn leading to Proud syndrome [5]. In vitro study demonstrated that these variants in NLS2 disrupted the normal nuclear localization of the ARX protein [5]. Another study illustrated that p.Arg332His, p.Arg332Pro and p.Thr333Asn mutants had a decreased transcription repression activity [6]. The p.Arg330His variant in this study has not been reported previously but it is also a conserved residue in NLS2. Besides, 3D modeling by STRUM implied that the variant caused fold destabilization of the ARX protein [4].

Our patient's epilepsy responded significantly but transiently to PLP which is a cofactor to catalyze the conversion of glutamate to GABA. In vitro studies of the *ARX* mutant suggested that *ARX*-associated phenotypes were resulted from the impairment of GABAergic neurons [7]. One of the direct targets of ARX is *SLC12A5* encoding the neuronal potassium-chloride cotransporter (KCC2) [8] which is a major extruder of intracellular chloride in neurons for subsequent chloride influx after GABA binding to its receptor for hyperpolarization and neuronal inhibition [9]. This implicated the role of ARX in controlling the responsiveness of neurons to GABA [8] and provided a possible explanation for the PLP responsiveness. Our finding suggested that *ARX* could be sequenced in patients with transient responsiveness to PLP, in addition to *PNPO* and *ALDH7A1* genetic defects.

Another interesting phenomenon was the elevation of alanine and lactate levels as well as muscle complex IV deficiency suggesting mitochondrial dysfunction in our patient. Muscle histology could be normal as reduction or loss of COX fibres might be indicative of mitochondrial DNA-related abnormality [10]. Clinically, he also had a phenotype resembling MD. Primary mitochondrial dysfunction could not be proven as no pathogenic variants were identified in genes encoding proteins associated with MDs or located in the mitochondrial proteome. Secondary mitochondrial dysfunctions can be caused by non-mitochondrial disease genes. However, no *ARX* genetic defect has been reported in patients presented with secondary mitochondrial dysfunction. A recent study showed that *TIMM8A* gene was one of the putative targets of ARX transcriptional activity [11]. Interestingly, *TIMM8A* encodes a protein located in the mitochondrial transmembrane space mediating the mitochondrial import system for metabolite carriers. *TIMM8A* mutations were associated with Mohr-Tranebjaerg syndrome which is a type of MDs and possibly resulted from a defect in mitochondrial oxidative phosphorylation owing to deficiency in carrier proteins [12,13]. The relationship of the present case of *ARX*-associated epileptic encephalopathy with primary mitochondrial disease is unclear and remains to be elucidated.

In conclusion, this is the first case report of *ARX*-associated infantile epileptic-dyskinetic encephalopathy which showed mitochondrial dysfunction and transient but complete responsiveness to PLP treatment. *ARX*-related encephalopathy is potential clinical mimicker of MD.

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Ethics

Ethical approval had been obtained from the Institutional Review Board (IRB) of the University of Hong Kong-Hong Kong West Cluster (IRB Ref. No.: UW 11-190). Written consent was obtained from the parents of the patient.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.braindev.2019.07.003.

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