Editorial

Fetal and neonatal MRI features of *ARX*-related lissencephaly presenting with neonatal refractory seizure disorder

Sara ffrench-Constant[1], Carolina Kachramanoglou[1], Brynmor Jones[1], Nigel Basheer[2], Nikolaos Syrmos[3], Mario Ganau[4], Wajanat Jan[1]

[1]Department of Imaging, [2]Department of Paediatrics, Imperial College Healthcare NHS Trust, London, UK; [3]School of Medicine, Aristotle University of Thessaloniki, Macedonia, Greece; [4]Department of Neurosurgery, Oxford University Hospitals NHS Foundation Trust, Oxford, UK

Correspondence to: Dr. Wajanat Jan, FRCR. Department of Imaging, Imperial College Healthcare NHS Trust, London, UK. Email: w.jan@nhs.net.

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Introduction

Classification systems for congenital brain malformations are usually based on the stage of brain development when these abnormalities occur, hence we commonly recognize malformations as the result of disorders of cell proliferation, neuronal migration and cortical organization (1). The most modern approaches to brain malformations are based on the identification of predictive biomarkers and specific genetic profiles meant to predict their clinical presentation and functional outcome (2). For instance, lissencephaly, a subtype of this heterogeneous group of disorders, is characterized by the absence of normal convolutions of the cerebral cortex and an abnormally small head; and numerous lissencephaly-related genes are currently known to cause it (3). Nonetheless, genetic confirmation of brain malformations can prove extremely time-costly and expensive hence clinicians and radiologists are constantly attempting to define precise fetal and neonatal imaging patterns which could potentially correlate specific phenotypes with their genetic mutations (4-9). To support the rationale for this approach, we present a case where the genetic profile of a lissencephaly caused by a mutation in the aristaless-related homeobox (*ARX*) gene was predicted solely by the precise identification of key features on prenatal and neonatal MRI. The *ARX* gene provides instructions for producing a protein that regulates the activity of other genes, it basically encodes a transcription factor involved in neuronal migration during the early stages of brain, testes, and muscle development. The specific aim of this pictorial case presentation is to demonstrate that state of the art imaging modalities allowed identification of the clues for *ARX* mutation during standard prenatal screening. Hence, setting the trend of ultra-early diagnosis may represent a relevant breakthrough in fetal and neonatal neuroimaging.

Exemplificative case

A couple of non-consanguineous parents, with no significant family history and two healthy children attended a routine appointment for antenatal US scan at 20 weeks of their third pregnancy. The mother (G3P2A0) was 27-years-old, and she reported that her pregnancy had been otherwise uneventful till that stage. The US unfortunately demonstrated an absent cavum septum pellucidum and suspected callosal agenesis. Given these concerning findings, another US scan was repeated at 25 weeks of gestation: this demonstrated abnormal sulcal pattern with thickened gyri in the temporoparietal regions. Of note, it was not possible to assess fetal gender in either scan. The couple was counselled to proceed with fetal MRI, which was performed at 26 weeks and confirmed the US findings but also demonstrating absence of the central sulcus and other primary sulci, in keeping with an imaging pattern of lissencephaly. There was relative prominent gyral folding of the medial temporal lobes. The neuronal eminence appeared to have remained as a mass of cells above the basal ganglia (see *Figure 1*). The parents were counselled but were committed to the pregnancy.

During the third trimester the mother reported abnormal fetal movements suggestive of seizure activity, and this raised the suspicions for difficult delivery by the team of

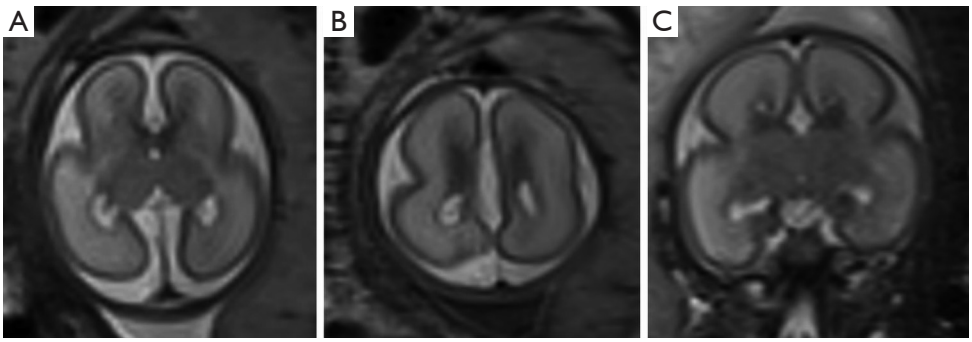

Figure 1 Fetal MRI at week 26 of gestation in the axial (A and B) and coronal planes (C) showing absent midline structures, poor sulcation and small ganglionic eminence.

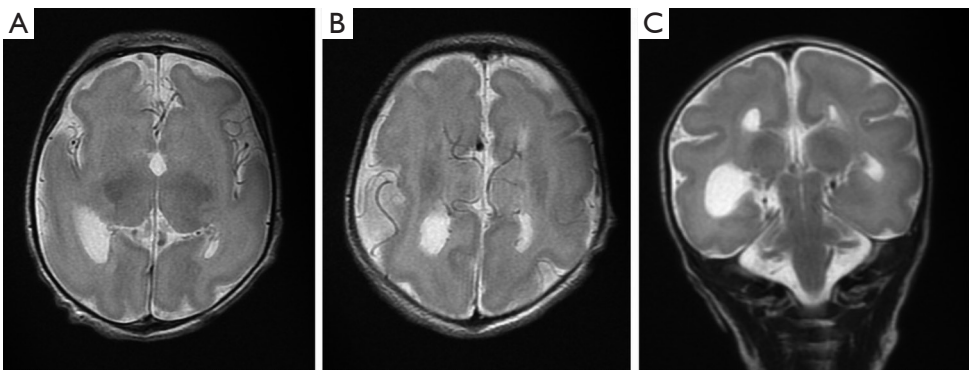

Figure 2 Neonatal MRI on day 4 of life in the axial (A and B) and coronal (C) planes showing small basal ganglia and lissencephaly with only relative thickening of the cortex. The imaging request form did not state the baby's gender.

obstetricians involved. The baby was born at 38 weeks and 3 days by vaginal delivery weighing 2.96 kg, with normal APGAR scores at time of birth and normal blood gases. Initial neonatal assessment confirmed tone abnormalities and ambiguous genitalia. Within the first few minutes of life, baby developed seizures and cerebral function monitoring (CFM) demonstrated persistent electrical seizure activity, refractory to multi-pharmacological management with antiepileptic medications (phenobarbitone and phenytoin in addition to levetiracetam).

Neonatal MRI performed on day 4 of life (see *Figure 2*) demonstrated microlissencephaly with agenesis of the corpus callosum and small indistinct basal ganglia but normal brainstem and cerebellum. Findings, in combination with the lack of patient's gender on the imaging request form, were consistent with X-linked lissencephaly with ambiguous genetalia (XLAG) in keeping with *ARX* mutation. Genetic testing indicated that the baby was a male, hemizygous for the *ARX c.994C>A p* mutation.

Discussion

In the past, congenital brain malformations were speculated to be relatively homogeneous clinical conditions, but as genetic, epigenetic and molecular research has progressed, the remarkable heterogeneity of these disorders has started to emerge. It is undeniable that the identification of specific causative genes, and subsequent studies shedding light on the pathophysiology cascade leading to specific phenotypes, has also led to giant leaps in the overall understanding of the development of the human brain. As for lissencephaly, multiple genetic mutations have been demonstrated and research in this field resulted in the identification of the so-called *LIS*-associated genes (10). The continuous advancement in the field of molecular genetics in the last decade has led to identification of at least 19 *LIS*-associated genes thus far (see *Table 1*), many of which are related to microtubule structural proteins (tubulin) or microtubule-associated proteins (MAPs) (4). These *LIS*-related

Table 1 Comprehensive list of LIS-associated genes (information extracted from Genetic Home Reference Database, United States National Library of Medicine https://ghr.nlm.nih.gov)

Class	Gene	Chromosomal location	Normal function	Health conditions related to genetic changes
Microtubule associated proteins (MAPs)-related genes	*PAFAH1B1/LIS1*	Ch 17; p13.3	Encodes for a subunit of a complex called platelet activating factor acetyl hydrolase 1B, which is involved in the structural organization of the neuronal cytoskeleton	• Isolated lissencephaly • Mille-Dieker syndrome • Subcortical band heterotopia
	DCX	Ch X; q23	Encodes for a protein called doublecortin involved in neuronal migration	• Isolated lissencephaly
	ARX	Ch X; p21.3	Encodes for a transcriptor factor involved in neuronal differentiation and migration	• X-linked lissencephaly with abnormal genitalia • Early infantile epileptic encephalopathy • Partington syndrome
	CDK6	Ch 7; q21.2	Encodes for a protein belonging to the family of serine/threonine kinases	• Microcephaly with lissencephaly
	RELN	Ch 7; q22.1	Encode for Reelin pathway-related proteins involved in many brain processes, including the extension of axons and dendrites	• Lissencephaly with cerebellar hypoplasia
	VLDLR	Ch 9; p24.2		
	NDEL1/NDE1	Ch 16; p13.11	Encode for Dynein-related proteins	• Lissencephaly type 4 • Microhydranencephaly • Charcot-Marie-Tooth syndrome
	DYNC1H1	Ch 14; q32.31		
	KIF5C	Ch 2; q23.1-q23.2	Encode for Kinesin-related proteins	• Cortical dysplasia complex with other brain malformations type 2 and 3
	KIF2A	Ch 5; q12.1		
Apoptosis-related genes	*CRADD*	Ch 12; q22	Encodes for a protein containing a death domain motif involved in the recruitment of caspase 2/ICH1 to the cell death signal transduction complex, and acts in promoting apoptosis	• Mental retardation, autosomal recessive 34, with variant lissencephaly
Tubulin-related genes	*TUBA1A*	Ch 12; q13.12	Encode for various protein involved in microtubule structure	• Lissencephaly with cerebellar hypoplasia • Isolated lissencephaly • Polymicrogyria with optic nerve hypoplasia • Cortical dysplasia complex with other brain malformations type 4, 6, 7, and 8
	TUBB2B	Ch 6; p25.2		
	TUBB3	Ch 16; q24.3		
	TUBG1	Ch 17; q21.2		
	TUBB3	Ch 16; q24.3		
	TUBG1	Ch 17; q21.2		
Actin isoform-related genes	*ACTB*	Ch 7; p22.1	Encode respectively for β- and γ-actin, both determining cell shape and controlling cell movement	• Baraitser-Winter syndrome
	ACTG1	Ch 17; q25.3		

genes include *LIS1*, *DCX*, *ACTB*, *ACTG1*, *ARX*, *CDK5*, *CRADD*, *DYNC1H1*, *KIF2A*, *KIF5C*, *NDE1/NDEL1*, *TUBA1A*, *TUBA8*, *TUBB*, *TUBB2B*, *TUBB3*, *TUBG1*, *RELN* and *VLDLR* (4). Among them, LIS-1 genotype is one of the commonest, and is characterized by a posterior predominance of lissencephaly with thickened cortex. By contrast, the double cortin (*DCX*) genotype demonstrates an anterior predominance or has a more diffuse appearance. Furthermore, lissencephaly may be associated with other craniofacial syndromes or callosal and brainstem abnormalities as well as cerebellar dysplasia (5).

The frequency and penetrance of the 19 known

genetic mutations related to lissencephaly is variable, nonetheless even the rarest ones tend to demonstrate specific phenotypic radiological and clinical appearances. This is in fact the case for *ARX* gene mutations which are a cause of X-linked lissencephaly with ambiguous genitalia (XLAG). In this rare mutation, the abnormal folding of cortical gyri displays a posterior predominance, with only relative mild cortical thickening. Additional phenotypical features of *ARX*-related lissencephaly are callosal agenesis and small basal ganglia. Clinically, the patients suffer from neonatal epilepsy, which tends to be medically-refractory despite multi-pharmacological treatment. In this form of lissencephaly, clonic convulsions or myoclonus tend to be seen from the first day of life, but neither infantile spasms nor hypsarrhythmia on electroencephalograms have been reported so far (6,11-14). Other symptoms described in the literature include lack of temperature control secondary to hypothalamic dysfunction, chronic diarrhoea secondary to pancreatic malfunction and cardiac abnormalities (15-17); although none of these were demonstrated in our patient. Overall prognosis is dismal, with the average life expectancy being around 18 months and a maximum recorded age of 4 years (18). This is the reason why we felt that this case represented the perfect example to stress the importance of identifying typical radiological features on prenatal US and MRI, highlighting that the finest diagnosis should be sought as soon as possible. The field of prenatal diagnosis based on in utero imaging is evolving at a rapid pace, so that many malformations involving any organ and apparatus (heart, lungs, bladder, genitalia, etc.) can nowadays be picked up at an ultra-early stage (19). Neuroimaging is certainly not lagging behind in this trend setting process.

The *ARX* gene is part of the homeobox family of proteins and plays an important role in embryonic development, interneuronal migration and forebrain differentiation. Crucially, protein products from the *ARX* gene also play a role in testicular development (20). Pathogenetic mutations in the *ARX* gene underlie a wide spectrum of phenotypes comprising many X-linked developmental disorders (21). Depending on the type of genetic mutation, phenotypes range from infantile spasms and mental retardation to lissencephaly and agenesis of the corpus callosum, as seen in XLAG. Hemizygous males are always severely affected, whereas female carriers may be unaffected or have a milder phenotype.

Kitamura *et al.* were the first to confirm that truncating or missense mutations of the *ARX* gene are linked to an XLAG phenotype in mice (22). These studies were followed up by screening for, and confirmation of, 'loss-of-function' *ARX* mutations in families with XLAG phenotypes (23,24). By contrast, other less severe *ARX*-related disorders were shown to be generally associated with hypomorphic and expansion mutations. Cho *et al.* further defined the pathogenesis of *ARX*-related disorders by demonstrating that *ARX* mutant cells have loss of DNA binding activity and reduced transcription repression activity when compared to wild type (25). The role of *ARX* in the generation and tangential migration of GABAergic neurons has been proven in mice models by knockout mutations resulting in the aberrant differentiation and migration of these neurons (26). Hence, the most affected in XLAG are the basal ganglia and ganglionic eminences, as demonstrated in this case. The cortex in an *ARX* brain is three-layered, as opposed to the usual six, and has been shown to have a significant lack of cortical GABAergic neurons, evidence supporting the role of ARX and the subsequent effects of its mutations (27). As detailed above, beside affecting cortical and neuronal development, *ARX* plays a crucial role in gonadal development. In mice, *ARX* expression is strong in fetal testis and *ARX* knockout mice have smaller testes and a decreased number of Leydig cells (28). The resultant decreased levels of testosterone are the principal cause for the genital ambiguity demonstrated in XLAG patients which can be demonstrated even during fetal US and MRI.

Conclusions

Although our knowledge of molecular mechanisms underlying brain development and causing congenital brain malformation is far from complete, a correlation between imaging patterns and genetic background can prove relevant in terms of prognostication. Even extremely rare cases, such as the one of X-linked lissencephaly secondary to an *ARX* mutation described here, can be accurately predicted with a careful assessment of the radiological features of prenatal US and MRI. An imaging diagnosis of XLAG is possible when under-development of the basal ganglia, agenesis of the corpus callosum and ambiguous genitalia are present in addition to lissencephaly. The neonatal refractory epilepsy is a major source of continuous hospital admissions and it is extremely detrimental for both the babies and their families. As such, attempts to identify specific radiological traits of these devastating neurological disorders and anticipate in utero diagnosis is important in order to provide proper counselling and accurate prognosis.

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None.

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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