European Journal of Medical Genetics 57 (2014) 284–287

Contents lists available at ScienceDirect

European Journal of Medical Genetics

journal homepage: http://www.elsevier.com/locate/ejmg

Clinical report

Mosaicism for c.431_454dup in *ARX* causes a mild Partington syndrome phenotype

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ARTICLE INFO

Article history:
Received 23 December 2013
Accepted 25 March 2014
Available online 13 April 2014

Keywords:
ARX
DNA sequencing
Duplication
Mosaicism
Partington syndrome
PCR fragment length analysis

ABSTRACT

A common in frame duplication in *ARX* (c.431_454dup24) was found in a five year-old boy who presented with mild Partington syndrome. The duplication was detected by PCR amplification followed by fragment length analysis and was located in exon 2 spanning the two polyalanine tracts commonly seen to expand. Detection of the duplication by DNA sequencing was difficult due to preferential sequencing of the normal allele, demonstrating the superiority of fragment length analysis in mosaic cases. The clinical symptoms were mild to moderate developmental delay with only the hand dystonia to suggest Partington syndrome. This patient is the first male reported to be mosaic for the duplication, and his clinical features are subtle. This study shows that in males with a phenotype of mild Partington syndrome and in heterozygous females fragment length analysis should be preferred over DNA sequencing.

1. Introduction

ARX (OMIM 300382) encodes a transcription factor of the Aristaless-related paired-class homeobox family. Mutations in *ARX* can cause non-syndromic mental retardation as well as syndromic forms with and without brain malformations, and at least 10 different clinical phenotypes have been described [Shoubridge et al., 2010]. Truncating mutations and some missense mutations cause syndromic forms with brain malformations including lissencephaly with abnormal genitalia (XLAG), Proud syndrome and agenesis of the corpus callosum. Expansions and duplications of the polyalanine tracts and also some missense mutations cause syndromic forms without malformations including the severe West syndrome (X-linked infantile spasm syndrome, ISSX), X-linked myoclonic epilepsy with spasticity and intellectual disability (ID) (XMESID), early infantile epileptic encephalopathy (EIEE), and the less severe Partington syndrome. Finally, non-syndromic X-linked ID can also be caused by mutations in *ARX* [Gecz et al., 2006; Guerrini et al., 2007].

The polyalanine tract at amino acid 144–155 in exon 2 is a mutational hotspot and the most common mutation in *ARX* is c.431_454dup24 (also assigned c.429_452dup24), causing an in frame duplication of the polyalanine tract by eight alanine residues p.(Ala148_Ala155dup). This mutation is present in approximately 40% of all unrelated patients with *ARX* mutations [Gecz et al., 2006]. The majority of patients with c.431_452dup24 display non-syndromic ID, while others display Partington syndrome. A minor part shows a severe phenotype of West syndrome [Shoubridge et al., 2010]. Partington syndrome (OMIM 309510) is an X-linked developmental disorder characterized by mild to moderate intellectual disability and variable movement disorders, especially hand dystonia.

In this report we present a young boy with a mild Partington syndrome phenotype and c.431_452dup24 in mosaic form. We hypothesize that some of the phenotypic variability seen in patients with c.431_452dup24 could be due to different levels of mosaicism. Furthermore, we emphasize that duplications especially of GC rich regions are difficult to detect by Sanger sequencing.

2. Clinical report

The proband is a five-year-old male, fourth child of healthy unrelated parents originating from Denmark. The mother was 30

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http://dx.doi.org/10.1016/j.ejmg.2014.03.009
1769-7212/

years and the father was 33 years at the time of the proband's birth. He had three older healthy sisters. Family history was negative. The couple had experienced 11 unexplained early pregnancy losses. The mother had parvovirus infection in the first trimester, but otherwise the pregnancy and delivery were uncomplicated. The boy was born at term, with a birth weight of 4000 g (+1.4 SD), a length of 55 cm (+1 SD) and head circumference of 35 cm (0 SD). Apgar score at 5 min was 10. Neonatal period was unremarkable but at 6 months of age, the parents began to suspect developmental delay. He walked at age 18 months and he spoke his first words at two years of age, and could speak in sentences of 4–5 words at four years of age. The boy was described as hypotonic and had reduced muscle strength. The boy awoke several times during the night from early childhood. He experienced recurrent acute otitis media and myringotomy tubes were inserted bilaterally at age two years. Adenoidectomy was performed simultaneously. This did not alter the sleep disturbance. Brain MRI and eye-exam performed at age four were normal.

When the boy was five years old he was referred to our department for genetic investigation of his developmental delay. Metabolic screening and molecular genetic investigation for fragile X syndrome were normal. Chromosome microarray analysis using Affymetrix CytoScan HD platform (Affymetrix, Santa Clara, USA) revealed a 36.5 kb maternally inherited deletion at 1p36.22, which was not considered to be the cause of the symptoms.

Clinical examination showed no dysmorphic features, and physical examination was unremarkable except for general hypermobility (Fig. 1). Growth parameters at 5 years of age were length 111.4 cm (−0.3 SD), weight 19.9 kg (+0.1 SD) and head circumference 52 cm (+0.2 SD). Social interaction, speech and motor skills were found by paediatric evaluation to be moderately delayed. He attends normal kindergarten, but has extra support. It was noticed that the boy had stereotypic movements of the shoulder and neck when excited, and that he often had fifth finger clenched below the 3rd or 4th finger (Fig. 1).

Based on these observations Partington syndrome was suspected and molecular genetic investigation of *ARX* was performed. Fragment chrX:25031634–25031846 (Human Genome Browser, hg19 build), located in exon 2 of *ARX*, was amplified using Expand Long Template Enzyme Mix (Roche Applied Science, Penzberg, Germany) in Failsafe J buffer (Epicentre Biotechnologies, Madison, WI, USA). This analysis showed amplification of two fragments of 231 bp and 241 bp respectively. The two peaks had approximately the same height and peak area (Fig. 2a). The 231 bp corresponds to the size of the fragment of a normal control while the 241 bp corresponds to the size of the peak in an individual with the duplication c.431_454dup. We then performed DNA sequencing using two primer sets amplifying region chrX:25031440–25032023 (primer set 1F-1R) and chrX:25031503–25031840 (primer set 3FH-3RH), respectively (Fig. 2b). DNA sequencing using primers 1F-1R showed no difference in sequence compared to the reference sequence. New primers were designed (3FH and 3RH) which were located in closer proximity to the c.431_454 region, and sequencing showed both wild type sequence and sequence containing the c.431_454dup, indicating that the duplication was present in mosaic form. A new blood sample was received from the proband, and repeated PCR fragment length analysis confirmed the result. PCR fragment length analysis of the mother showed only wild type sequence indicating that the mutation was *de novo*.

Fig. 1. Photo of the proband at age 5, demonstrating abnormal finger positioning.

Evaluation of the boy at age 5 years and 8 months by a physiotherapist showed affected fine motor skills, reduced functional endurance and joint hypermobility (Beighton score 8/9). The boy performed a 6 min walk test in which he walked significantly below the age-expected distance indicating a low endurance [Lammers et al., 2008; Ulrich et al., 2013]. He was assessed with the Movement Assessment Battery for Children – Second edition (MABC-2) [Schoemaker et al., 2012] which showed a normal result. However, he used a very immature pencil grip; he had no hand preference and was unable to reproduce shapes or letters. Parents filled in the Pediatric Evaluation of Disability Inventory – Computer Adaptive Test (PEDI-CAT) [Dumas and Fragala-Pinkham, 2012; Dumas et al., 2010] in which the normative scores fell within the lower end of the normal range in all four areas. The boy had difficulties performing daily living tasks which require finger strength and precision such as buttoning and zipping during dressing and using utensils during mealtime.

The study followed the tenets of the Declaration of Helsinki and was approved by the local medical ethics committee. The family was informed of the nature of the study and written informed consent was obtained.

3. Discussion

The mosaic c.431_454dup duplication was detected in PCR fragment length analysis and was only visible at DNA sequencing after design of primers located closely to the duplication. This phenomenon has been observed previously by our group for *CYP27B1* where a duplication went undetected in heterozygous carriers (unpublished data). This shows the limitations of DNA sequencing in detecting duplications when present in heterozygous or mosaic form, especially if the duplications are GC rich in base composition, and underscores the importance of using PCR fragment length analysis in investigation of Partington syndrome.

Duplication of the polyalanine tract in exon 2 (encoding amino acids 144–155) of *ARX* has been observed to cause a broad clinical spectrum from severe to mild ID, with or without infantile spasms or other forms of epilepsy, and/or hand dystonia. Both inter – and intrafamilial variability has been observed [Cossee et al., 2011]. The patient described in this study had dystonic movements of the hands suggestive of Partington syndrome. Otherwise he was only mildly affected with regards to fine and gross motor skills and everyday functional skills. Motor and language milestones were mildly delayed but as yet no formal IQ testing has been carried out. The relatively mild phenotype observed in this patient is probably due to the duplication c.431_454dup being present in mosaic form. The mother does not carry the mutation indicating that the mutation arose *de novo* after zygote formation. We have only investigated the blood and hence do not know the extent of mosaicism. It could be hypothesized that some of the phenotypic