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SHORT COMMUNICATION

Mutational screening of *ARX* gene in Brazilian males with mental retardation of unknown etiology

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Abstract *ARX* gene mutations have been known as important causes of developmental and neurological disorders and are responsible for a large spectrum of abnormal phenotypes, includeing syndromic as well as nonsyndromic forms of mental retardation. We have screened the entire coding and flanking intronic sequences of *ARX* gene in 143 mentally impaired males in order to investigate the contribution of *ARX* mutations to mental retardation in the population of Rio de Janeiro, Brazil. Three sequence variants were identified: one patient had the most recurrent mutation already observed in *ARX* gene, the c.428_451dup(24 bp), two patients presented the c.1347C>T (p.G449G) in exon 4, and one patient had the intronic variant c.1074-3T>C. Although two of these alterations were considered polymorphisms, the known pathogenic variant c.428_451dup(24 bp) was found at a high rate (4.8%) among X-linked mental retardation (XLMR) families. Our results, the first in Latin America, reinforce the idea that *ARX* mutations are relevant to mental retardation and are indicative that molecular screening of exon 2 should be considered in males with mental retardation of unknown etiology, associated or not with neurological manifestations, especially in familial cases.

Keywords *ARX* · X-linked mental retardation · Epilepsy · Dystonia

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Introduction

The A*ristaless-related homeobox* (*ARX*) gene (MIM 300382) is a homeobox-containing gene; it maps to Xp22.13, encodes a protein of 562 amino acids, and acts as a transcription factor (Stromme et al. 2002). The expression pattern suggests that *ARX* is involved in the differentiation and maintenance of specific neuronal cell types in the central nervous system (Ohira et al. 2002; Poirier et al. 2004; Suri 2005).

ARX mutations are responsible for a wide spectrum of phenotypes, including various forms of epilepsy, myoclonic seizures, dystonia, autism, lissencephaly, as well as syndromic and nonsyndromic X-linked mental retardation (Bienvenu et al. 2002; Kitamura et al. 2002; Stromme et al. 2002; Poirier et al. 2006). Previous studies estimated that *ARX* mutations are highly prevalent among X-linked mental retardation (XLMR) families (9.5%), with approximately 6.5% due to a c.428_451dup(24 bp) mutation, which suggests an important role for this gene in XLMR (Mandel and Chelly 2004; Poirier et al. 2006). In this work, we report the mutational screening of the entire *ARX* gene in Brazilian males with mental retardation (MR) of unknown etiology.

Materials and methods

We screened a total of 143 unrelated males from Rio de Janeiro, Brazil, aged 2–29 years (10.2±5.3), with mild to severe MR associated or not with other clinical features (Table 1) for *ARX* mutations. The patients were classified as XLMR families (with at least two affected males in a kindred who are related through females), BP families (with at least two affected male siblings), and sporadic cases (SC). This included 66 syndromic (11 XLMR, 6 BP, and 49 SC) and 77 non-syndromic (10 XLMR, 8 BP, and 59 SC) mentally retarded individuals. Chromosomal analysis and fragile-X syndrome test had normal results. The regional Ethics Committee approved the research protocols, and informed consent was obtained from the parents of patients.

Genomic DNA was obtained from peripheral blood (Miller et al. 1988), and mutation screening of all five exons and flanking intronic regions of *ARX* was performed using PCR, followed by single-strand conformation polymorphism (SSCP) analysis (exons 1, 3, 4, and 5) and direct sequencing (exon 2). PCR conditions are available on request. The primer pairs used were previously designed by the group of Dr. Jozef Gécz and are available on request (Stromme et al. 2002).

The splice site predictions were carried out using the electronic tools: http://www.fruitfly.org/seq_tools/splice.html (Berkley Drosophila Genome Project); http://www.tigr.org/tdb/GeneSplicer/gene_spl.html (Gene Splicer Web Interface); http://www.cbs.dtu.dk/services/NetGene2/ (NetGene2 Server); http://www.l25.itba.mi.cnr.it/~webgene/wwwspliceview.html (Institute of Advanced Biomedical Technologies). For exonic splicing enhancers (ESE) predictions we used the http://www.rulai.cshl.edu/tools/ESE/ (Cartegni et al. 2003), and RESCUE-ESE at http://www.genes.mit.edu/burgelab/rescue-ese (Fairbrother et al. 2002).

Results

Mutational analysis of the *ARX* gene in 143 Brazilian mentally retarded males revealed one case of the mutation c.428_451dup(24 bp) in a male with a familial history of XLMR (Fig. 1), one exonic variant in two sporadic MR cases, and a novel intronic variant in a male with sporadic MR. No variants were identified in exons 1, 3, and 5 (Table 2).

The patient with the mutation c.428_451dup(24 bp) has moderate MR, language delay, macrocephaly, and prominent forehead. This mutation was also observed in the proband's older brother, and in his cousin, both mentally retarded males. The family's clinical features have been previously detailed by us (Gestinari-Duarte et al. 2006).

In two other patients we found a silent variant in exon 4 (c.1347C>T, p.G449G): the first, a 7-year-old boy, presenting with MR, seizures/epilepsy, and autism, and the second, a 15-year-old, presenting with MR and language delay. We also identified the c.1347C>T mutation in 2.6% (2/78) of our sample of normal controls.

A novel intronic variant (c.1074-3T>C) was identified in one patient, a 9-year-old boy with moderate MR, seizures, and language delay. This variant is located 1 bp upstream of the 3′ splice site of *ARX* intron 2. His mother was found to be a carrier of the variant; however, we did not observe this substitution in his nonaffected sister. This variation was predicted not to alter splicing, and it could not be associated with the etiology of MR. We found the same intronic variant in approximately 1% (1/102) of normal controls.

Table 1 Clinical description of the study sample

Main clinical features	XLMR families		BP families		Sporadic cases		Total (%)
	S	NS	S	NS	S	NS	
MR and autism	1	2	0	2	15	10	30 (21)
MR and epilepsy/seizures	3	3	2	2	11	12	33 (23.1)
MR, autism, and epilepsy/seizures	1	1	1	1	1	4	9 (6.3)
MR associated or not with other clinical features	6	4	3	3	22	33	71 (49.6)
Total (%)	11	10	6	8	49	59	143
	21 (14.7)		14 (9.8)		108 (75.5)		

XLMR families: families with at least two affected males in a kindred who are related through females; BP families: families with at least two affected male siblings; sporadic cases: cases with MR

S Syndromic MR, *NS* nonsyndromic MR

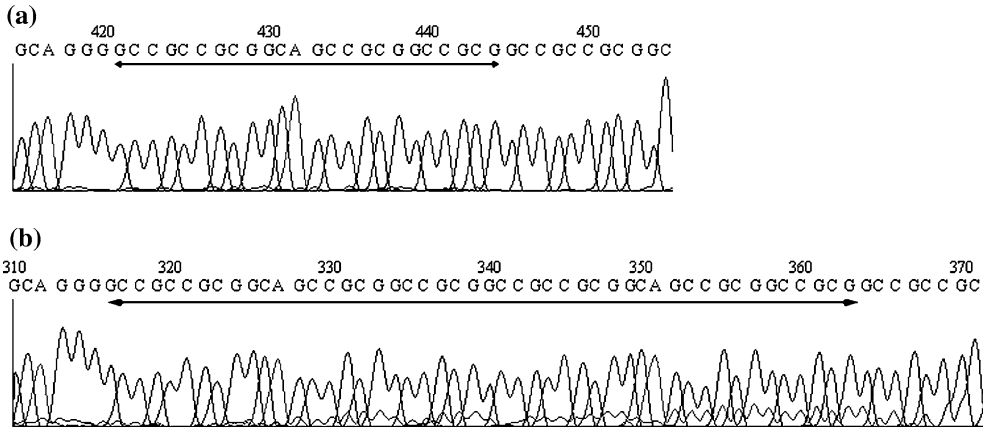

Fig. 1a, b DNA sequence electropherograms from exon 2 of *ARX* showing the 428_451dup(24 bp) mutation. **a** Normal control, **b** affected male

Table 2 Summary of the *ARX* sequence variants detected in 143 patients with MR from Brazil

Variant number	Nucleotide change	Amino acid change	Frequency in this study
1	c.428_451dup (24 bp)	A (12>20) expansion	1
2	c.1347C>T	p.G449G	2
3	c.1074-3T>C	Intronic	1

Discussion

ARX gene mutations have already been considered as an important cause of syndromic and nonsyndromic XLMR (Mandel and Chelly 2004; Ropers and Hamel 2005). Our study is the first *ARX* screening in MR males from Latin America, and contributes new clues about the significance of this gene for genetic counseling of individuals with MR of unknown etiology.

The most common *ARX* mutation is the c.428_451dup(24 bp), which leads to an elongation of the second poly A tract in ARX protein (Stromme et al. 2002). This mutation was reported in Partington syndrome, West syndrome, and in approximately 70% of MRX families linked to Xp22.1 (Bienvenu et al. 2002; Ropers et al. 2003). The data showed a high frequency (6.1%) of the c.428_451dup(24 bp) in XLMR families and a low rate in BP families (1.5%) (Poirier et al. 2006). In comparison, we found one male, a familial case, with this duplication among 21 XLMR families (4.8%). We did not find this mutation in 14 BP families or in 108 SC. To date, 31 families with the c.428_451dup(24 bp) mutation including those from this report, have been described (Bienvenu et al. 2002; Frints et al. 2002; Stromme et al. 2002; Turner et al. 2002; Gronskov et al. 2004; Kato et al. 2004; Partington et al. 2004; van Esch et al. 2004; Gestinari-Duarte et al. 2006; Poirier et al. 2005, 2006; Stepp et al. 2005; Nawara et al. 2006).

The silent variant c.1347C>T was identified in two boys with MR, and in 2.6% of our normal population, and has been considered a polymorphism. Although this variant was firstly identified in a boy with XLAG (Kato et al. 2004) and in a boy with MR (Gronskov et al. 2004), it was also found in 4% of the normal controls tested in these studies. Gronskov et al. (2004) performed in-silico analysis through ESEfinder program and showed that c.1347C>T creates a strong binding site for SRp55, a kind of splicing binding factor to exonic splice enhancers (ESE). However, in our study, using the RESCUE-ESE program, this same analysis did not show clearly possible ESE sites. The ability of both programs to predict natural point mutations that disrupt genuine ESE motifs has yet to be fully assessed (Baralle and Baralle 2005).

We excluded a pathogenic role of the novel intronic variant c.1074-3T>C. This substitution was identified in a boy with MR and in 1% of our normal controls. Our results did not shown any significant alterations in the splicing site values compared to the wild-type sequence, which suggests that it is a polymorphism. To date, 11 intronic variants have been reported in the *ARX* gene, 9 as polymorphisms and 2 have been identified in XLAG individuals, and described as premature stop codon mutations (Kato et al. 2004).

In conclusion, our data showed that the 24-bp duplication in exon 2 of the *ARX* gene has a considerable frequency among XLMR families, but the incidence of this mutation in sporadic males with MR appears to be low, reinforcing previous suggestions (Poirier et al. 2006; Stepp et al. 2005) that *ARX* mutation screening of exon 2 should be considered for any male with MR of unknown etiology, particularly among familial cases. Recently, mutations in *CDKL5*, also located within the Xp22, have been associated with a broad range of phenotypes, such as seizures/epilepsy, autism, and MR (Kalscheuer et al. 2003; Mari

et al. 2005; Scala et al. 2005), which overlap the cases for *ARX* mutations. We believe that *CDKL5* is, perhaps, another gene that should be screened in patients presenting MR associated with seizures/epilepsy and autism, particularly in XLMR families, who have negative results for *ARX* mutations.

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References

Baralle D, Baralle M (2005) Splicing in action: assessing disease causing sequence changes. J Med Genet 42:737–748

Bienvenu T, Poirier K, Friocourt G, Bahi N, Beaumont D, Fauchereau F et al (2002) *ARX*, a novel Prd-class-homeobox gene highly expressed in the telencephalon, is mutated in X-linked mental retardation. Hum Mol Genet 11:981–991

Cartegni L, Wang J, Zhu Z, Zhang MQ, Krainer AR (2003) ESEfinder: a web resource to identify exonic splicing enhancers. Nucleic Acids Res 31:3568–3571

van Esch H, Poirier K, de Zegher F, Holvoet M, Bienvenu T, Chelly J et al (2004) *ARX* mutation in a boy with transsphenoidal encephalocele and hypopituitarism. Clin Genet 65:503–505

Fairbrother WG, Yeh RF, Sharp PA, Burge CB (2002) Predictive identification of exonic splicing enhancers in human genes. Science 297:1007–1013

Frints SG, Froyen G, Marynen P, Willekens D, Legius E, Fryns JP (2002) Re-evaluation of MRX36 family after discovery of an *ARX* gene mutation reveals mild neurological features of Partington syndrome. Am J Med Genet 112:427–428

Gestinari-Duarte RS, Santos-Rebouças CB, Boy RT, Pimentel MMG (2006) *ARX* mutation c.428_451dup (24 bp) in a Brazilian family with X-linked mental retardation. Eur J Med Genet 49(3):269–275

Gronskov K, Hjalgrim H, Nielsen IM, Brondum-Nielsen K (2004) Screening of the *ARX* gene in 682 retarded males. Eur J Hum Genet 12:701–705

Kalscheuer VM, Tao J, Donnelly A, Hollway G, Schwinger E, Kubart S et al (2003) Disruption of the serine/threonine kinase 9 gene causes severe X-linked infantile spasms and mental retardation. Am J Hum Genet 72:1401–1411

Kato M, Das S, Petras K, Kitamura K, Morohashi K, Abuelo DN et al (2004) Mutations of *ARX* are associated with striking pleiotropy and consistent genotype-phenotype correlation. Hum Mutat 23:147–159

Kitamura K, Yanazawa M, Sugiyama N, Miura H, Iizuka-Kogo A, Kusaka M et al (2002) Mutation of *ARX* causes abnormal development of forebrain and testes in mice and X-linked lissencephaly with abnormal genitalia in humans. Nat Genet 32:359–369

Mandel JL, Chelly J (2004) Monogenic X-linked mental retardation: is it as frequent as currently estimated? The paradox of the *ARX* (Aristaless X) mutations. Eur J Hum Genet 12:689–696

Mari F, Azimonti S, Bertani I, Bolognese F, Colombo E, Caselli R et al (2005) CDKL5 belongs to the same molecular pathway of MeCP2 and it is responsible for the early-onset seizure variant of Rett syndrome. Hum Mol Genet 14:1935–1946

Miller SA, Dykes DD, Polesky HF (1988) A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 16:1215

Nawara M, Szczaluba K, Poirier K, Chrzanowska K, Pilch J, Bal J et al (2006) The ARX mutations: a frequent cause of X-linked mental retardation. Am J Med Genet 140A:727–732

Ohira R, Zhang YH, Guo W, Dipple K, Shih SL, Doerr J et al (2002) Human *ARX* gene: genomic characterization and expression. Mol Genet Metab 77:179–188

Partington MW, Turner G, Boyle J, Gecz J (2004) Three new families with X-linked mental retardation caused by the 428–451dup(24 bp) mutation in *ARX*. Clin Genet 66:39–45

Poirier K, van Esch H, Friocourt G, Saillour Y, Bahi N, Backer S et al (2004) Neuroanatomical distribution of *ARX* in brain and its localisation in GABAergic neurons. Brain Res Mol Brain Res 122:35–46

Poirier K, Abriol J, Souville I, Laroche-Raynaud C, Beldjord C, Gilbert B et al (2005) Maternal mosaicism for mutations in the *ARX* gene in a family with X linked mental retardation. Hum Genet 118:45–48

Poirier K, Lacombe D, Gilbert-Dussardier B, Raynaud M, Desportes V, de Brouwer AP et al (2006) Screening of ARX in mental retardation families: consequences for the strategy of molecular diagnosis. Neurogenetics 7(1):39–46

Ropers HH, Hamel BC (2005) X-linked mental retardation. Nat Rev Genet 6:46–57

Ropers HH, Hoeltzenbein M, Kalscheuer V, Yntema H, Hamel B, Fryns JP et al (2003) Nonsyndromic X-linked mental retardation: where are the missing mutations? Trends Genet 19:316–320

Scala E, Ariani F, Mari F, Caselli R, Pescucci C, Longo I et al (2005) CDKL5/STK9 is mutated in Rett syndrome variant with infantile spasms. J Med Genet 42:103–107

Stepp ML, Cason AL, Finnis M, Mangelsdorf M, Holinski-Feder E, Macgregor D et al (2005) XLMR in MRX families 29, 32, 33 and 38 results from the dup24 mutation in the *ARX* (Aristaless related homeobox) gene. BMC Med Genet 6:16

Stromme P, Mangelsdorf ME, Shaw MA, Lower KM, Lewis SM, Bruyere H et al (2002) Mutations in the human ortholog of Aristaless cause X-linked mental retardation and epilepsy. Nat Genet 30:441–445

Suri M (2005) The phenotypic spectrum of *ARX* mutations. Dev Med Child Neurol 47:133–137

Turner G, Partington M, Kerr B, Mangelsdorf M, Gecz J (2002) Variable expression of mental retardation, autism, seizures, and dystonic hand movements in two families with an identical *ARX* gene mutation. Am J Med Genet 112:405–411