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Clinical Report

Asplenia in ATR-X Syndrome: A Second Report

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Mutation at the ATR-X locus is associated with severe mental retardation. Several conditions, initially reported as clinically distinct phenotypes, have now been attributed to ATR-X mutation. Asplenia, in association with severe mental retardation, has been reported and subsequently demonstrated in one family to be due to ATR-X mutation. We now report on a second instance of a patient presenting with mental retardation and asplenia who has been shown to have a mutation at the ATR-X locus.

KEY WORDS: ATR-X; asplenia; pneumococcal meningitis; Howell–Jolly bodies

INTRODUCTION

The syndrome of alpha thalassaemia and mental retardation (ATR-X) dates from the 1981 report of Weatherall et al. [1981] of mentally-retarded children with hemoglobin H disease, subsequently elaborated by Wilkie et al. [1990a,b] into an X-linked form and a chromosome 16-linked form with associated abnormality of the alpha globin gene complex. Gibbons et al. [1991] and Wilkie et al. [1991] offered a more complete description of the X-linked form, emphasizing the hallmark characteristics of severe mental retardation, microcephaly, growth retardation, characteristic facial gestalt, and hemoglobin H inclusion bodies seen by staining with 1% brilliant cresyl blue. Further reports contributed to the emergence of a wider phenotype, including male pseudohermaphroditism [Reardon et al., 1995]. Subsequently mutations have been identified in the *ATRX* gene on Xq13 [Gibbons et al., 1995] and, to date, in excess of 60 mutations have been reported in 97 families [Gibbons and Wada, 2004].

Following the initial reporting of *ATRX* mutations in ATR-X, it quickly became apparent that several other phenotypes, which had been reported as clinically distinct were also due to mutation at the *ATRX* locus. Such instances of clinical disorders allelic to ATR-X include Juberg–Marsidi syndrome [Villard et al., 1996a], Chudley–Lowry syndrome [Chudley et al., 2002], Holmes–Gang syndrome [Stevenson et al., 2000], Carpenter–Waziri syndrome [Abidi et al., 1999], and mental retardation without hematological evidence of HbH inclusion bodies [Villard et al., 1996b; Gibbons and Higgs, 2000].

The entity known as Smith–Fineman–Myers syndrome stems from the 1980 report of brothers with mental retardation, seizures, neurological dysfunction, and short stature [Smith et al., 1980]. Subsequent cases considered to have the same disorder have been reported by Stephenson and Johnson [1985], Wei et al. [1993], Guion-Almeida et al. [1998], and Adès et al. [1991]. In general, the facial features of subjects reported have not been noteworthy, with the exception of the Ades et al. report. As suggested by Hall [1992], the facial features of the brothers reported by Ades were reminiscent of ATR-X syndrome. Although HbH inclusion bodies were not seen in the family [Adès, 1992], mutation analysis has subsequently demonstrated an acceptor splice site mutation in exon 34 of the *ATRX* gene [Villard et al., 2000].

A specific clinical aspect of one of the brothers reported by Adès et al. [1991] was absence of the spleen. This is an uncommon finding and certainly not well-observed in ATR-X or allelic phenotypes. Indeed, asplenia has not been observed in a large cohort of individuals (>150) with all clinical variants of ATR-X and allelic forms [Gibbons, personal observation, 2005]. Obviously, most such patients have not been specifically examined ultrasonographically for asplenia, and no data are available on asplenia-associated infective problems in these patients. We now report on a second patient with *ATRX* mutation and asplenia whose clinical presentation has involved recurrent pneumococcal infections.

CLINICAL REPORT

The patient was delivered at 37 weeks gestation by emergency caesarian for breech presentation with fetal distress. He is the third child and second son of his nonconsanguineous parents. There is no family history of X-linked mental retardation. His birthweight was 2,670 g (25th centile) and his Apgar scores were 6 at 1 and 10 at 5 min, respectively.

While the pregnancy was uneventful, the patient's mother reported reduced fetal movements retrospectively. He initially required hospitalization for poor feeding and failure to thrive. When he returned for his 6-week check, he was found to have persistent hypotonia, poor head control, inability to fix and follow, and had no social smile. His parents found him to be very irritable, with prolonged bouts of inconsolable crying. He also suffered myoclonic-jerking episodes. This persisted throughout infancy. An EEG performed at 2 months of age revealed brief episodes of epileptic activity during sleep. He was commenced on anticonvulsants for his seizures. Routine cytogenetics confirmed a normal male, 46XY, karyotype.

The patient had poor weight gain. His feeding difficulties continued. There were recurrent bouts of aspiration pneumonia, and had a PEG tube inserted at 9 months of age. Throughout his first and second year, he had multiple admissions with poor seizure control, worsening irritability, and recurrent respiratory tract infections. He made slow neurodevelopmental progress. At age 1 year, the patient suffered an episode of pneumococcal sepsis, and at 23 months of age, he was admitted with pneumococcal sepsis and meningitis. A blood film at this

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time revealed Howell–Jolly bodies, and no spleen could be visualized on abdominal ultrasound. The patient was commenced on prophylactic ampicillin, and covered with pneumococcal and quadrivalent meningococcal vaccination. He has had no further episodes of invasive pneumococcal disease.

Evaluated by a geneticist, at age 3 years, extreme developmental delay was noted. Facial features observed were of small, posteriorly rotated, low set ears with over-folded helices and a left sided pre-auricular pit, downslanted palpebral fissures and hypertelorism with a broad flat nasal bridge, a short philtrum with a tented upper lip, small teeth with widely spaced upper central incisors, and a patulous lower lip (Fig. 1). His palate was normal apart from hypertrophy of the palatal ridges. The hands and fingers were short, with single palmar creases bilaterally. He had pronounced kyphosis. Neurologically, his early hypotonia had been replaced by hypertonia with brisk reflexes, especially in the lower limbs, which are held in hyperextension with his feet plantar flexed. His external genitalia were normal.

At 3 years and 4 months of age, the patient used three words with meaning. Ophthalmological examination was normal aside from a mild right-sided divergent squint. He could crawl, pull to stand, and cruise along the furniture. He had a good pincer grasp, and could transfer objects from hand to hand. He was very sociable and affectionate, and demonstrated no stranger anxiety. He was not capable of independent feeding. He was not toilet trained. An MRI brain scan performed at 3 years of age revealed increased T2-weighted signal intensity within the white matter of the centrum semi-ovale, deep peritrigonal white matter, and peripherally in the frontal white matter.

Specialized investigations included staining for HbH inclusions, which was normal on two occasions. Genomic DNA was extracted from the buffy coat of blood collected in EDTA by a standard phenol chloroform method. The ADD domain was investigated by sequence analysis and the helicase and 3′end of the gene was analyzed by denaturing high-performance liquid chromatography (WAVE^{TM}) and then by sequence analysis [Steensma et al., 2004]. The region commonly mutated in the ADD domain was normal. For WAVE analysis, patients's amplicons were cross-hybridized with normal amplified DNA. A single fragment, spanning exon 32, showed an abnormal WAVE pattern (Fig. 2A). Sequence analysis of this fragment identified an A6811 > G mutation (Fig. 2B) that would result in a R2271G change. The nucleotide change was confirmed in a second sample from the patient and his mother was found to be a carrier for this mutation. This change is not observed in control samples and is not previously recorded in the *ATR-X* mutation spectrum. Moreover, this residue is conserved in both mouse and chicken, the obvious conclusion being that nature has not tolerated mutation at this residue.

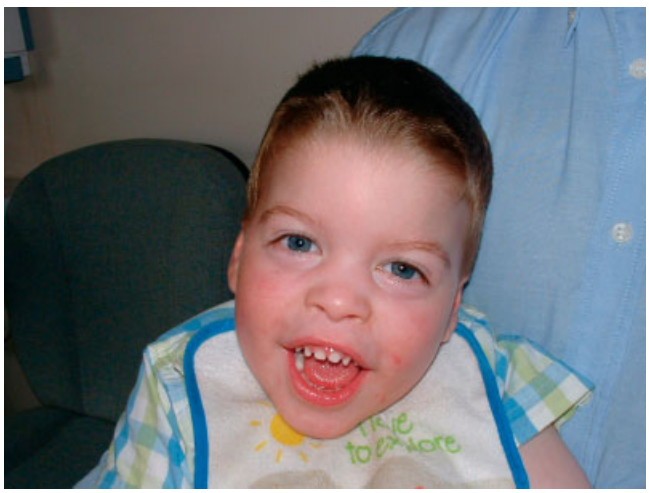

Fig. 1. The typical facial features of ATR-X are shown. Note especially the rather small teeth.

DISCUSSION

The patient, we describe, shows facial characteristics and a clinical course entirely in keeping with the well-established phenotype of ATR-X and the identification of a mutation at the *ATRX* locus is not surprising. However, the asplenia is unusual, interesting, and worthy of further consideration. The recurrent pneumococcal infections are entirely consistent with the asplenia.

One patient has been recorded with *ATRX* mutation and asplenia [Villard et al., 2000]. That patient had originally been reported as a likely case of Smith–Fineman–Myers syndrome [Adès et al., 1991]. It is by no means certain that all reports of Smith–Fineman–Myers syndrome reported, to date, share the same underlying genetic entity. [Smith et al., 1980; Stephenson and Johnson, 1985; Adès et al., 1991; Wei et al., 1993; Guion-Almeida et al., 1998]. As with our own patient, Adès et al. [1991] observed Howell–Jolly bodies in the peripheral blood film of Case 1 of that report, leading to a diagnosis of congenital asplenia. Howell–Jolly bodies are spherical blue-black inclusions of red blood cells seen on Wright-stained smears. They are nuclear fragments of condensed DNA, 1–

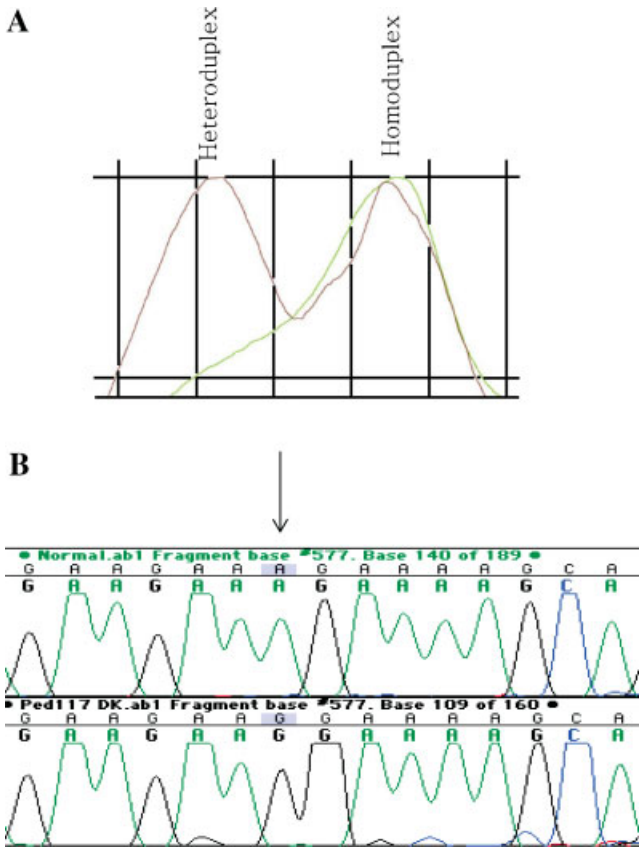

Fig. 2. **A**:WAVE^{TM} analysis after normalization against the major peak of exon 32 in the patient compared against a normal control run at 57.6°C. Primer sequences available upon request. **B**: Sequence chromatogram showing the *A* GA → *G* GA point mutation in codon 2271 of the *ATRX* gene using the forward amplification oligonucleotide.

2 μm in diameter, normally removed by the spleen. They are seen in severe hemolytic anemias, in patients with dysfunctional spleens or after splenectomy. Asplenia impairs opsonization of encapsulated organisms, leaving the individual vulnerable to invasive pneumococcal and meningococcal disease. This is evident from our clinical report, which describes two such episodes in the first 2 years of the patient's life, both episodes requiring intensive care management. Lifelong antibiotic prophylaxis and additional vaccination cover against pneumococcus and meningococcus can reduce the incidence of sepsis in asplenic patients. Interestingly, Case 1 reported by Adès et al. [1991] was not recorded as having suffered such infectious episodes. In common with the patient we report, Case 1 reported by Adès et al., did not have HbH inclusion bodies on staining with brilliant cresyl blue [Adès, 1992]. However, as has since become apparent, there is a 15% false negative rate in identifying patients with ATR-X in screening with this method [Gibbons, personal observation, 2005].

Asplenia was not a consistent finding in the two brothers reported by Adès et al. [1991], the second brother not having this feature. Neither has absence of the spleen been recorded in other reports of Smith–Fineman–Myers syndrome. However, the cases of Adès et al. differ from the other reports in that mutation of the *ATRX* gene has been sought and identified in these boys [Villard et al., 2000]. An exon 35 acceptor splice site mutation in these brothers resulted in a frame shift [Villard et al., 2000]. In contrast, we record an exon 32 missense mutation, resulting in amino acid substitution resulting in the asplenia associated ATR-X phenotype in our patient.

Asplenia is not observed in ATR-X phenotype generally. Experience encompassing in excess of 150 established cases has not identified a single previous instance [Gibbons, personal observation, 2005], though it is acknowledged that most patients will not have had specific investigations for absence of the spleen. Neither is it recorded that there is an increased propensity of pneumococcal or meningococcal infections in ATR-X patients, which might be attributable to the asplenia.

Since asplenia seems to be such an uncommon feature of the ATR-X phenotype, an alternative consideration would be that the asplenia in this patient and in Case 1 of Adès et al. [1991] is a manifestation of heterotaxy and that mutation at *ATRX* may promote heterotaxy. There is little evidence for such from extensive phenotypic studies of patients with confirmed *ATRX* mutation. Gibbons and Higgs [2000] have reviewed the clinical spectrum of features in 145 cases from 80 families, only one instance of dextrocardia being observed and no other features recorded, which might clinically suggest a heterotaxia. One is driven, by the absence of evidence to the contrary, to conclude that asplenia is a very occasional finding in the ATR-X phenotype, but which can be, as this cases demonstrates, associated with potentially catastrophic and life threatening infectious consequences.

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