



## SHORT REPORT

# Germline and gonosomal mosaicism in the ATR-X syndrome

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**We have identified two females who are mosaic for an *ATR-X* mutation. One case, in whom the mutation was undetectable in peripheral blood and buccal cells, has two affected sons and is therefore presumed to be a germline mosaic. In another case, the *ATR-X* mutation is weakly detectable in the peripheral blood but only one of her three children who share the disease-associated haplotype carries the mutation and therefore it is concluded that she is a gonosomal mosaic. These cases provide the first molecular evidence for the occurrence of post-zygotic mutation in X-linked  $\alpha$  thalassaemia mental retardation syndrome. The possibility of germline mosaicism must therefore be considered in the genetic counselling of ATR-X families.**

**Keywords:** ATR-X syndrome; XLMR; *ATR-X* mutation; germline mosaicism

## Introduction

X linked  $\alpha$  thalassaemia mental retardation syndrome (ATR-X) is a rare disorder characterised in male patients by severe mental retardation, a typical facial appearance, genital abnormalities and  $\alpha$  thalassaemia.<sup>1</sup> In this X-linked recessive disorder, the biological fitness in affected individuals is zero and, as expected, new mutations are frequently encountered in affected families. Therefore, when counselling the parents of sporadic cases and assessing the risk of recurrence, the ability to identify female carriers is of great importance.

In ATR-X syndrome, female carriers are intellectually normal. Donnai *et al*<sup>2</sup> reported hypertelorism and midface hypoplasia in a mother and daughter who were proven carriers. However, in over 70 other families

there is no indication that facial changes or any other associated phenotypic abnormalities are present in female carriers (unpublished data). Nevertheless, in approximately 25% of obligate carriers, rare cells with HbH inclusions (the hallmark of  $\alpha$  thalassaemia) may be found.<sup>1</sup> Studies of the pattern of X inactivation in carriers for ATR-X show that the mutant X chromosome is predominantly inactivated in cells from a variety of tissues, explaining the scarcity of HbH inclusions in these individuals.<sup>3</sup> The presence of HbH inclusions and a highly skewed pattern of X inactivation has, in the past, been used to identify female carriers in affected families. The former test, however, lacks sensitivity and the latter specificity as 5–10% females in general population have skewed X inactivation. Characterisation of the underlying mutation greatly facilitates the identification of carriers and if the mother is negative for the mutation the residual risk depends on the likelihood of germline mosaicism.

*ATR-X*, the gene mutated in ATR-X patients, has been identified.<sup>4</sup> Characterisation of the underlying mutation in affected boys has allowed 'definitive' testing of females in 60 families. This has revealed two females who are mosaic for the *ATR-X* mutation. One

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case, in whom the mutation was undetectable in the somatic tissues tested, has two affected sons and is therefore presumed to be a germline mosaic. In another case the *ATR-X* mutation is weakly detectable in the peripheral blood but only one of her three children who share the disease-associated haplotype carries the mutation and therefore it is concluded that she is a gonosomal mosaic, ie both somatic and germline mosaicism is present. These observations allow an estimate to be made of the frequency of germline mosaicism in ATR-X syndrome.

## Patients and Methods

### Families

The affected males in the families described have the classical features associated with ATR-X syndrome (pedigree 11 (Figure 1) and pedigree 19 (Figure 2<sup>1</sup>). In pedigree 19, two males (2-1,2-4) were born prematurely and died soon after birth. No further clinical information is available.

### Haplotype Analysis

Polymorphic markers *DXS559*, *DXS227*, *DXS1225*, *DXS986* and *DXS995* (details via Genome Database <http://www.gdb.org>) were analysed by PCR and polyacrylamide electrophoresis.

### Mutation Analysis

The mutations in pedigrees 11 and 19 have been described previously.<sup>5</sup> The numbering adopted in this report is based on U75653; the addition of eight bases at the 5' end corrects this to the transcriptional start site.<sup>6</sup> In pedigree 11 the mutation 961G > A destroys a *StyI* restriction site CCTTGG and creates an *MboI* restriction site GATC. Genomic DNA from family members was amplified by PCR using primers xnp127 (5'-ATCCATCGCTCGAAACTCGG-3') and PHD1 (5'-AGCCGTGACTCAGATGGAATGGATG-3'). Aliquots of the product were digested either with *StyI* or *MboI* (New England Biolabs (UK) Ltd, Hitchin, Hertfordshire, UK) and the fragments resolved by electrophoresis in 1% agarose.

In pedigree 19 the mutation (934G > T) was detected using allele-specific oligonucleotide hybridisation. Oligonucleotide probes were designed to hybridise preferentially to wildtype or mutant sequence as described in.<sup>7</sup> Genomic DNA from family members was amplified by PCR using primers xpn103 (5'-TATTGTGGACAACCTCTTTCGACC-3') and xnp126 (5'-TCCTGGAGATTTCAG-3'). The PCR fragments were electrophoresed on 1% agarose and blotted onto Hybond-N<sup>+</sup> membrane (Amersham Life Science Ltd, Little Chalfont, Buckinghamshire, UK). Oligonucleotides (wild-type; 5'-CATTTCTTGCAGAAAGCA-3', mutant; 5'-TGCTTTCTTCAAGAAATG-3') were labelled with [<sup>32</sup>P] dCTP and hybridised to the membrane as described by Oldridge *et al.*<sup>8</sup>

### Methylation Studies

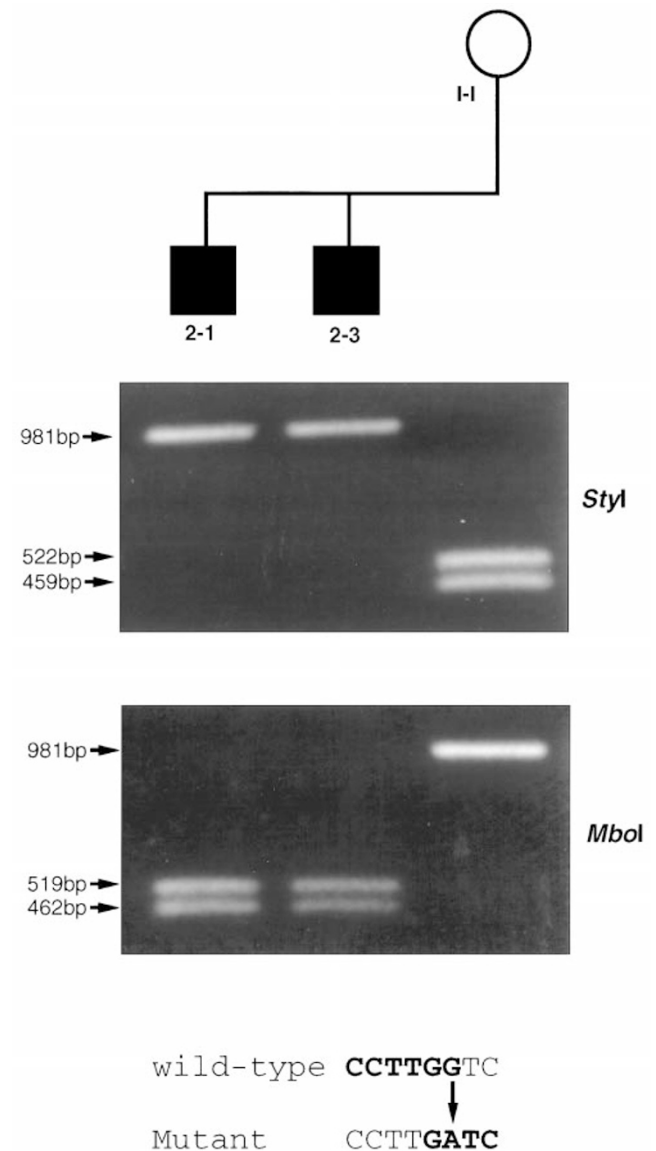
To assess the pattern of X-inactivation, methylation was analysed at the loci *PGKI* and *DXS255*. Methylation studies were carried out on DNA from female carriers digested with the isoschizomers *MspI* (methylation insensitive) and *HpaII*

(methylation sensitive) and probed either with pSPT-PGK (*PGKI*) or M27β (*DXS255*) as previously described.<sup>3</sup>

## Results

### Pedigree 11

The mutation in the affected boys is 961G > A which in this case is not in the context of a highly mutable CpG



**Figure 1** Restriction enzyme analysis of the 981bp xnp127/PHD1 PCR product in pedigree 11. The affected boys are shown by closed squares, the mother by an open circle. Digestion with *StyI* cleaves the wild-type fragment; digestion with *MboI* cleaves the mutant fragment. In the lower figure, the context of the G → A mutation is shown; the enzyme sites are in bold

sequence (Figure 1). The mutation destroys a *StyI* restriction site (CCTTGG) and creates an *MboI* site (GATC). This mutation, however, was absent in DNA from the mother's leukocytes (Figure 1) and buccal cells (data not shown). Serial dilutions were used to show that, by this method, the mutation would be detectable at 1 part in 64.

### Pedigree 19

Haplotype analysis demonstrated that the affected patient's sisters (2-2 and 2-3) had inherited the disease-associated haplotype (Figure 2). The pattern of X inactivation was determined by studying methylation status at loci *DXS255* (for 1-1 and 2-2) and *PGKI* (for 2-3). Using this analysis, we have previously shown that carriers of ATR-X have a skewed pattern of X inactivation (>90:10) with the mutant X predominantly inactivated. In the females studied here we showed balanced patterns of X inactivation in 1-1 and 2-2 but moderately skewed X inactivation (about 80:20) in 2-3. However, in this case, contrary to the pattern found in ATR-X carriers, the X chromosome with the disease-associated haplotype was predominately active (data not shown).

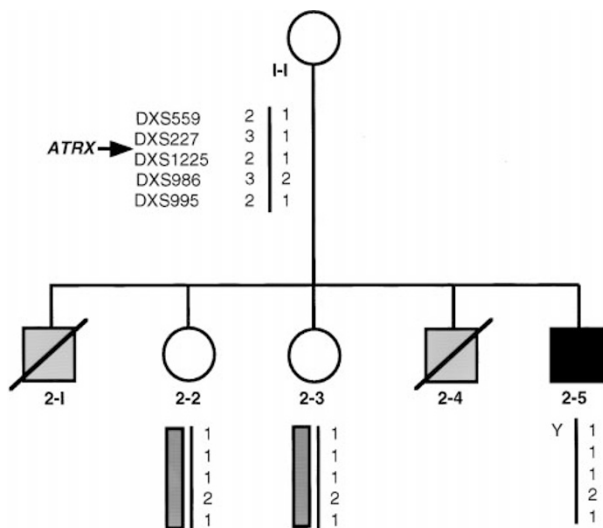
No red cells with HbH inclusions were identified in the three females. On the basis of these findings it seemed likely that the mutation in the affected male occurred as a *de novo* event. Direct mutation analysis (Figure 3), however, revealed a weak positive signal for

the mother (1-1) although both sisters (2-2, 2-3) were negative for the mutation. Serial dilutions showed that the signal in the mother was 1/64 of that in the proband (2-5). Repeated analysis with fresh dilutions of the mother's DNA sample gave identical results. The possibility of contamination of the maternal DNA sample with the patient's was considered unlikely since the DNA extraction of blood samples had been carried out on separate occasions. A fresh maternal sample was not available.

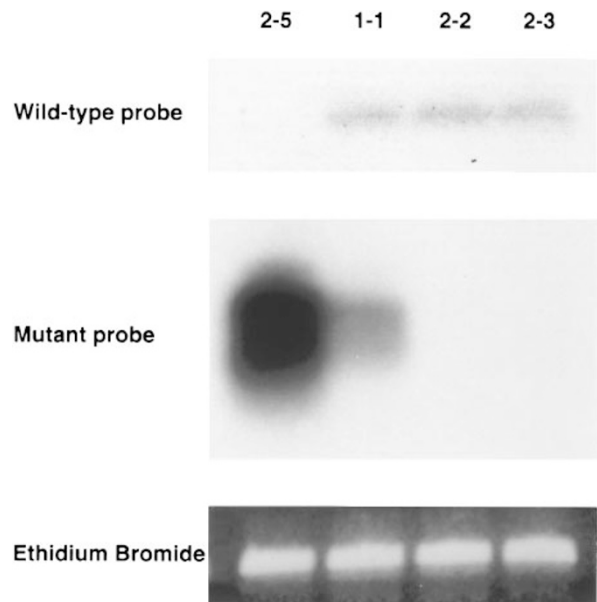
### Discussion

Two families are presented with evidence of maternal mosaicism for a mutation in *ATRX*. In pedigree 11, the mother has two affected boys but the underlying mutation is undetectable in two somatic tissues tested (leukocytes and buccal cells) and it is possible that the mosaicism is restricted to the germline. In contrast, in pedigree 19, a mutation is detectable in the mother but absent from two sisters who share their affected brother's disease haplotype but appear not to carry the disease. This indicates that the mother is a gonosomal mosaic.

We have identified mutations in 60 families; three involve >1 kb deletions, three involve insertions or



**Figure 2** Pedigree 19 showing haplotypes: the order of the loci from centomere to Xqter is DXS559, DXS227, ATRX, DXS1225, DXS986, DXS995. The two male sibs, who were born prematurely and died soon after birth are indicated by shaded squares



**Figure 3** Allele specific oligo hybridisation using either wild-type or mutant specific probes against amplified DNA from pedigree 19 (see Figure 2). In the lower figure is the original gel, stained with ethidium bromide, to show equivalence of loading

deletions of 1-2 bps, 54 are single base substitutions (manuscript in preparation). In 23 families there are affected males in different generations or branches of the pedigree and it is clear that the mothers of the probands are obligate carriers. In six cases material from the mother is not available for testing. In 31 families, where there is a sporadic case (20 families) or affected sibs (11 families), the mothers have all been tested for the presence of the mutation (summarised in Figure 4). There is evidence of maternal mosaicism in two families. Considering that many of the families in this study are small or have unaffected children too young for genetic testing, these figures may underestimate the frequency of mosaicism. For example, in singleton families the presence of germline mosaicism would not be apparent.

The principal issue when counselling is the risk of recurrence for families with a sporadic case of ATR-X. In this study, 17/20 mothers of sporadic cases were shown to be carriers of ATR-X by mutation analysis and this high figure may reflect a higher male than female mutation rate. Based on this experience, in families where the mutation has not been identified, we would expect 85% (17/20) of the mothers of sporadic cases to be carriers. Additional information may be sought by examining for the presence of HbH inclusions and studying the pattern of X inactivation (see above). If the mutation has been identified, then based on this small set of data, the risk of germline carriage in a mother testing negative for the mutation is 25% (1/4).

In diseases where new mutations are frequent it is important, particularly for counselling the parents of sporadic cases, to know the likelihood of germline mosaicism. Zlotogora, in a review of the literature,<sup>9</sup> distinguished two groups of diseases: group 1 in which somatic and germline mosaicism in one of the unaf-

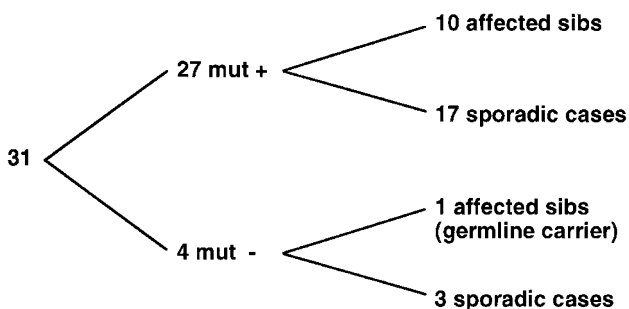
ected parents is relatively frequent, and group 2 in which mosaicism is almost never found. ATR-X joins the group of conditions, eg fascioscapulohumeral dystrophy, haemophilia, Christmas disease, osteogenesis imperfecta and Duchenne muscular dystrophy, where post-zygotic mutation is relatively 'common'<sup>9</sup> and germline mosaicism is an important consideration in the counselling of parents of sporadic cases when the mutation is not detected in the mother.

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**Figure 4** Summary of sporadic cases and affected sibs with ATR-X syndrome and the breakdown of cases born to mothers who test positive (mut +) or negative (mut-) for an ATRX mutation