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Mutation of the XNP/ATR-X Gene in a Family with Severe Mental Retardation, Spastic Paraplegia and Skewed Pattern of X Inactivation: Demonstration that the Mutation is Involved in the Inactivation Bias

To the Editor:

A family in which severe mental retardation (MR) is segregating with spastic paraplegia (SP) has recently been reported (Martinez et al. 1998). The extended pedigree of this family is presented in figure 1. Obligate-carrier females have a totally skewed pattern of X inactivation, detected by amplification of the $(CAG)_n$ microsatellite repeat in the androgen-receptor gene and previous digestion of genomic DNA with the methylation-sensitive restriction endonuclease *Hpa*II, as described elsewhere (Martinez et al. 1998). Genetic analysis in the family has revealed linkage of the morbid locus to the proximal long arm of the X chromosome, with a maximum LOD score in Xq13.3. Three genes involved in X-linked MR (XLMR) have already been reported in this genomic region. One, encoding oligophrenin-1, is involved in nonsyndromic XLMR (Billuart et al. 1998) and thus does not seem to be a good candidate. The other two are involved in two syndromic XLMR conditions: Menkes syndrome (MIM 309400) and alpha-thalassemia with mental retardation (ATR-X [MIM 301040]) syndrome. The latter is an XLMR condition that associates severe MR, mild alpha-thalassemia, typical facial dysmorphy and a skewed pattern of X inactivation in carrier females (Gibbons et al. 1995*a*). The clinical characteristics of the reported MR+SP family are close to this description, and thus the presence of an allelic mutation at the ATR-X locus could be hypothesized. In addition to the already reported clinical features in the MR+SP family, hematologic analysis revealed that 3% of the patients' erythrocytes showed HbH inclusions after cresyl-brilliant staining, which reinforced the possibility that a mutation in the XNP/ATR-X gene is present in this family. However, ATR-X syndrome has always been reported to be associated with neonatal hypotonia, which can be severe (Gibbons et al. 1995*a*). In the case of the family that we studied, affected

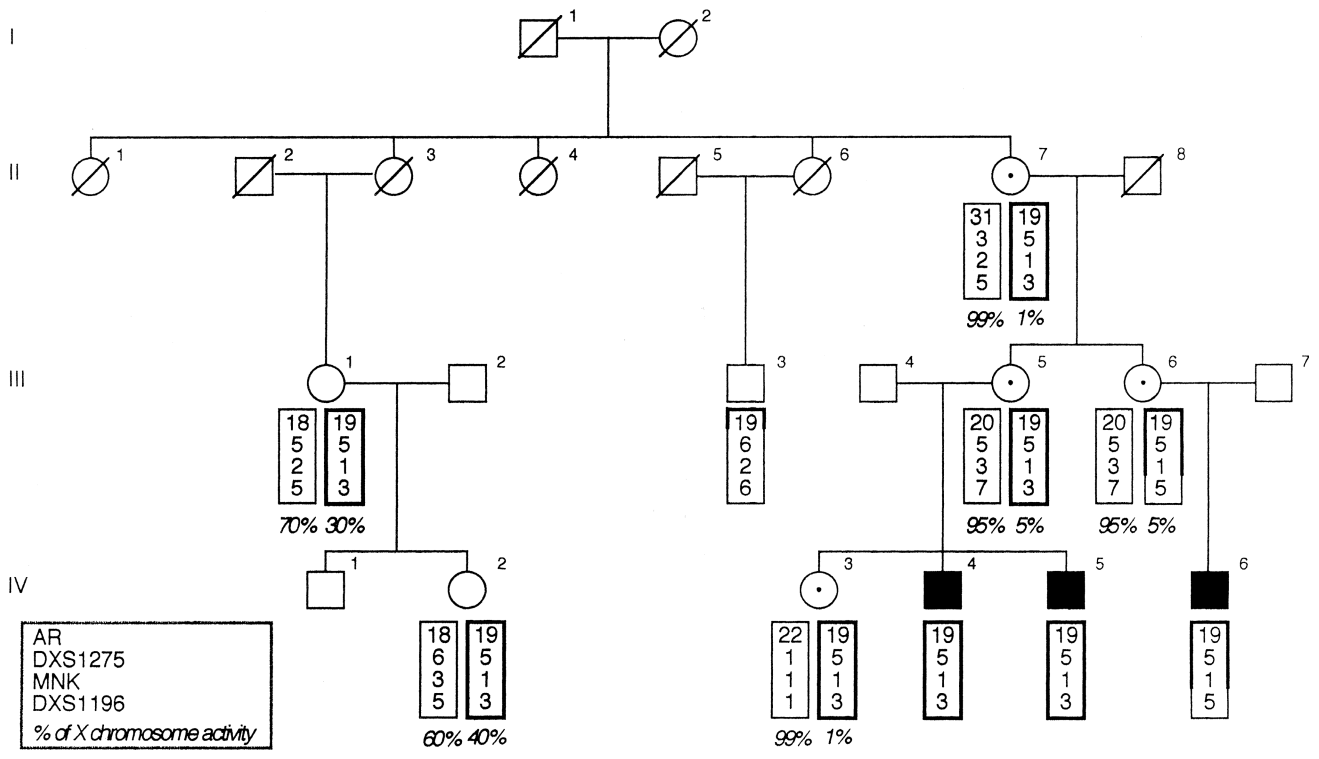

Figure 1 Pedigree of family showing the haplotypes (shown below each analyzed member) of the region linked to the disease. The order of the markers in the region, with respect to the X inactivation–specific transcript (XIST gene) and the XNP/ATR-X gene, is AR-DXS1275-XIST-XNP-MNK-DXS1196. Note that III-1 and IV-2 have inherited the disease-linked haplotype (shown in the thicker frames). The percentages of X inactivation in females are shown (in italics) under the corresponding haplotype (for further details, see text).

patients were affected, from birth, with the opposite sign, hypertonia. It was thus interesting to look for a possible new mutation that might lead to a variant of the disorder.

The gene involved in the ATR-X syndrome was isolated in 1994 (by Gecz et al.) and named "XNP." It has since been shown to be mutated in 13 patients with alpha-thalassemia MR (ATR-X) syndrome (Gibbons et al. 1995*b*). Moreover, mutations in this gene have also been identified in families affected with Juberg-Marsidi syndrome (MIM 309590) and with MR syndromes without alpha-thalassemia (Villard et al. 1996*a*, 1996*b*, 1996*c*). More recently, a new mutation has been found, in Carpenter-Waziri syndrome (Abidi et al. 1999).

The gene is large (probably extending over 300 kb) and consists of 35 exons. It encodes a putative zinc-finger helicase (Villard et al. 1997) that is probably involved, by remodeling the structure of the chromatin, in the control of gene expression. This assumption is supported by the finding of an interaction between the XNP protein and the Ezh2 protein (Cardoso et al. 1998), the human equivalent of *Drosophila* enhancer of zeste, a chromatinian protein of the Polycomb group.

To date, 34 different mutations in 50 pedigrees have been described in the XNP/ATR-X gene, which are located either in the region coding for the three zinc-finger domains of the protein (exons 7 and 8 and the beginning of exon 9) or in the helicase domain, which extends over 3 kb at the COOH terminus of the putative protein.

We thus decided to search for mutations in this gene in a patient from the MR+SP family. This was performed by systematic sequencing of the gene. We found one mutation in the gene, changing an arginine from the conserved helicase domain III into a lysine, R1742K (fig. 2). This mutation perfectly segregates with the disorder in the family (fig. 3). Moreover, the mutation was absent from 100 unrelated chromosomes from the same geographical area as that of the family we studied. We performed reverse-transcription PCR (RT-PCR) on RNA from lymphocytes of the patients, to check for any putative effect of the mutation on the mRNA splicing, which has been previously reported to occur in this gene (Villard et al. 1996*b*) but we failed to find any abnormal-size product (data not shown).

Since this amino acid change (arginine to lysine) is generally considered to be conservative, we used a local structure–prediction program (PEPTIDESTRUCTURE, from the GCG package, which mainly predicts α helices

Figure 2 Position of mutation in the MR+SP family. The top two lines show the normal XNP protein and nucleotide sequence encompassing part of the helicase III domain. The sequences are numbered according to GenBank entry U75653. The R1742 residue is underlined, as is the G5459 nucleotide that is changed into an A and that causes the replacement of R1742 by K (both the normal and mutated codons are shaded).

and β sheets). This program has predicted a change in the local structure of the protein (fig. 4), potentially arising because of steric problems. Arginine 1742 is an amino acid that cannot be strictly considered to be evolutionarily conserved, but it is present at the same position in several members of the helicase family of proteins (e.g., in the yeast Rad54 protein).

Very interestingly, in exploring another branch of the family for the presence of the mutation, we found two females (individuals III-1 and IV-2 in fig. 1) who share the disease-associated haplotype, spanning both the XNP gene and the X-inactivation center, but who lack the R1742K mutation. We thus can deduce that the mutation arose de novo in the germline of one member of the founding couple (I-1 or I-2 in fig. 1). Furthermore, the fact that females in the nonmutated branch of the family do not exhibit a skewed pattern of X-chromosome inactivation demonstrates that the skewing in carrier females is directly linked to the presence of the mutation in this gene. This is the first demonstration of a direct link between a mutation in this gene and the skewed pattern of X inactivation observed in all carrier females, and it further supports the pathogenicity of the R1742K mutation.

We can draw, from these data, two main conclusions. First, the analysis of this family extends the phenotype associated with XNP/ATR-X mutations. All patients reported so far were hypotonic (at least in the neonatal period), which made this trait a major criterion of inclusion. In this family, patients are hypertonic and also present spastic paraplegia. This clinical sign has never been observed either in classic ATR-X patients or in related phenotypes such as Juberg-Marsidi syndrome or Carpenter-Waziri syndrome. Spasticity can develop at later stages in ATR-X patients, despite neonatal hypotonia, but the patients reported in this study were affected with spasticity from birth. We have no explanation for this, except that no mutation affecting the helicase III conserved domain has been identified so far. However, it is difficult to draw conclusions based on the analysis of a single family.

In contrast, a reexamination of the affected males in this family shows that, although one affected male does not exhibit a markedly dysmorphic phenotype, the two other affected individuals present some facial features in common with the ones observed in ATR-X syndrome: everted lower lip with a "carplike" triangular mouth, hypertelorism, small triangular nose, and broad nasal root.

The second point regards the skewed pattern of X inactivation in carrier females. So far, only a close association between a bias in X inactivation and a mutation in the gene has been reported, without any direct proof. In this family, we can observe that the bias in X inactivation occurs only in females carrying the mutation. Other females, who have received the same chromosome but who are not mutated at the XNP locus, do not present a skewed pattern of X inactivation, demonstrating that this phenomenon is directly related to a mutation in the gene. We do not have an explanation for this phenomenon, but we can postulate two hypotheses. First, a mutation in the gene could lead to a selection, during embryogenesis, that favors the cells expressing the normal gene product. The second hypothesis is that the gene, which is involved in chromatin-structure remodeling (Cardoso et al. 1998), could participate directly or indirectly in one of the processes of

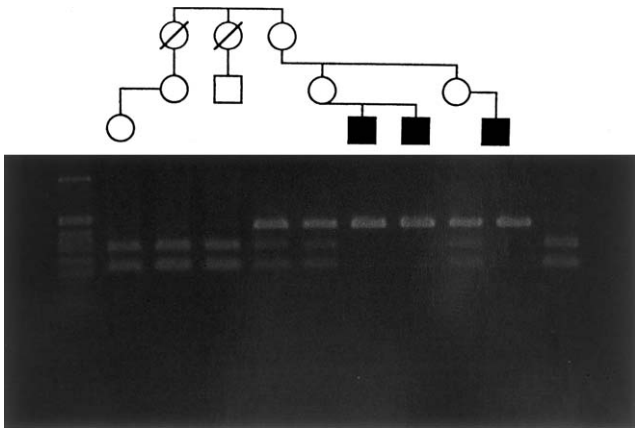

Figure 3 Detection of 5459G→A mutation in the affected branch of the family. A PCR product obtained by use of two oligonucleotides flanking the mutation is digested with *Bse*RI and electrophoresed. The presence of the mutation destroys an internal *Bse*RI site in the amplification product. Affected males have a single undigested amplified allele (one band); carrier-female DNA harboring both a mutated and a nonmutated allele yields both a digested and a nondigested PCR product (three bands); and noncarrier females have two digested alleles (two bands).

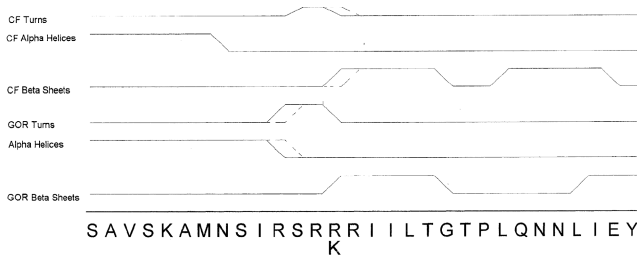

Figure 4 Prediction of secondary structures of the normal (*continuous line*) and the mutated (*dashed line*) proteins. CF = secondary-structure prediction according to the Chou-Fasman algorithm; GOR = the Garnier-Osguthorpe-Robson algorithm. Note the variation for Turns and β-sheet formation (with CF method) and for Turns and α-helix formation (with GOR method), in the region surrounding the R1742K substitution.

X-chromosome inactivation: either spreading or maintenance of the inactive state. This would be lethal in females in whom the mutated allele is expressed, because functional disomy of the X chromosome is known to be a lethal condition. Whatever the truth is, it is really striking to observe that a gene defect that leads to such a strong counterselection in female carriers and that is not restricted to certain cell lineages does not impair male viability. It is, to our knowledge, the first instance in which such a negative selection against the cells expressing an abnormal gene product in females does not imply a male-lethal condition (discussed in Pegoraro et al. 1997). Although highly skewed X inactivation has been reported in several other X-linked disorders, it is never systematically present in the obligate-carrier females (Orstavik et al. 1998; Plenge et al. 1999).

A final point is that the bias in X inactivation is, together with severe MR, the only consistent sign in families. This can certainly be important in a first clinical screening of the patients, before a mutation is sought in the gene.

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Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

Human Gene Mutation Database (HGMD), http://www.uwcm.ac.uk/uwcm/mg/search/136052.html (for the mutations reported in the XNP/ATR-X gene)

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim (for ATR-X, Juberg-Marsidi, and Menkes syndromes)

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A 22q11.2 Deletion That Excludes *UFD1L* and *CDC45L* in a Patient with Conotruncal and Craniofacial Defects

To the Editor:

Microdeletions of chromosome 22q11.2 occur with a high frequency in the general population, with an estimated incidence of 1/3,000–1/4,000 (Burn and Goodship 1996). They have been shown to be associated with the malformation phenotypes of velocardiofacial syndrome (VCFS [MIM 192430]), DiGeorge syndrome (DGS [MIM 188400]), and conotruncal anomaly face syndrome (CAFS [MIM 217095]) (Emanuel et al. 1999*a*). Deletions of this region have also been demonstrated in some patients with the autosomal dominant form of Opitz G/BBB syndrome (MIM 145410) (McDonald-McGinn et al. 1995). Significant phenotypic overlap is found among these entities, including conotruncal cardiac defects, craniofacial anomalies, learning disabilities, and cleft palate. The spectrum of clinical findings shows considerable variability, even within families (McLean et al. 1993).

Although the overwhelming majority (>85%) of patients have deletions of the same ~3-Mb region (Emanuel et al. 1999*a*), several reports have described patients with atypical, shorter deleted segments nested within the large typically deleted region (TDR) (Levy et al. 1995; Kurahashi et al. 1996; O'Donnell et al. 1997; McQuade et al. 1999). Recently, a small, 20-kb deletion within the TDR was reported in a patient with a classic VCFS/DGS phenotype. This smaller deletion disrupts the *UFD1L* and *CDC45L* genes, the products of which (in particular, *UFD1L*) have been suggested to play important roles in craniofacial and cardiac development resulting in the phenotype (Shaikh et al. 1999; Yamagishi et al. 1999). However, several of the aforementioned patients (some of whom have cardiac and craniofacial defects) have deletions that do not include the region containing these genes. These observations suggest that additional sequences within the TDR affect early craniofacial and cardiac morphogenesis. Additionally, a patient with features of DGS and with a microdeletion that falls outside the TDR but that does not overlap with any of the known deletions was recently described (Rauch et al. 1999). This patient had craniofacial abnormalities and an interrupted aortic arch (type B) with truncus arteriosus, the same defect seen in the patient described by Yamagishi et al. (1999). The report by Rauch et al. further emphasizes the likelihood that the 22q11.2-related cardiac defects are unlikely to result from defects involving a single gene within the TDR.

We have identified a patient, CH98-18 (Emanuel et al. 1998), with a novel deletion of chromosome 22q11.2. His deletion is distal to the usual 3-Mb deletion found in most patients with VCFS and appears to overlap with a portion of the deleted region described by Rauch et al. (1999). The deletion does not overlap with any of the previously described "minimal critical regions" for VCFS/DGS. The patient was born to a 33-year-old mother at 35 wk gestation. The pregnancy was complicated by a weight gain of 70 lbs and premature rupture of membranes. The baby was delivered by cesarean section, because of breech presentation, with Apgar scores of 7 at 1 min and 8 at 5 min. Physical examination at birth was notable for an appropriate-for-gestational-age infant with hypertelorism, posteriorly rotated ears, micrognathia, a loud cardiac murmur, hypospadias, descended testes, single palmar creases, and 5th-finger clinodactyly bilaterally. Renal and cranial ultrasounds were normal. Echocardiography showed the presence of truncus arteriosus type II and a ventricular septal defect. Borderline hypocalcemia was also present. The patient had surgical repair of his truncus arteriosus at age 3 wk and a replacement graft at age 7 mo.

Motor development was normal. The patient sat at age 6 mo and walked at age 14 mo. However, he had expressive-speech delay, speaking his first words at age 21 mo. At age 26 mo, he had speech appropriate for an 18-month-old. During a recent physical examination at age 26 mo (fig. 1), short stature, microcephaly, a prominent glabella, partially attached earlobes, a broad nasal bridge, a broad nasal tip with a crease, hypoplastic nasal alae, anteverted nares, a featureless philtrum, a downturned mouth, a bifid uvula, and normal hearing and vision were noted. Endocrine evaluation including thyroid-function and growth-hormone panels was unremarkable. Immunologic studies including surface markers for T-cell, B-cell, and NK lineages, myeloid markers, leukocyte adhesion, and Wiskott-Aldrich–associated