

Report

Transcription Factor *SOX3* Is Involved in X-Linked Mental Retardation with Growth Hormone Deficiency

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Physical mapping of the breakpoints of a pericentric inversion of the X chromosome (46,X,inv[X][p21q27]) in a female patient with mild mental retardation revealed localization of the Xp breakpoint in the *IL1RAPL* gene at Xp21.3 and the Xq breakpoint near the *SOX3* gene (SRY [sex determining region Y]-box 3) (GenBank accession number NM_005634) at Xq26.3. Because carrier females with microdeletion in the *IL1RAPL* gene do not present any abnormal phenotype, we focused on the Xq breakpoint. However, we were unable to confirm the involvement of *SOX3* in the mental retardation in this female patient. To validate *SOX3* as an X-linked mental retardation (XLMR) gene, we performed mutation analyses in families with XLMR whose causative gene mapped to Xq26-q27. We show here that the *SOX3* gene is involved in a large family in which affected individuals have mental retardation and growth hormone deficiency. The mutation results in an in-frame duplication of 33 bp encoding for 11 alanines in a polyalanine tract of the *SOX3* gene. The expression pattern during neural and pituitary development suggests that dysfunction of the *SOX3* protein caused by the polyalanine expansion might disturb transcription pathways and the regulation of genes involved in cellular processes and functions required for cognitive and pituitary development.

X-linked mental retardation (XLMR) affects ~1/600 males, with a carrier frequency of 2.4/1,000 in females (Herbst and Miller 1980), and is often highly heterogeneous. It can be classified into (i) specific forms (MRXS), in which mental retardation is associated with facial or growth abnormalities and/or metabolic or neuromuscular disorders, and (ii) nonspecific forms (MRX), in which affected patients have no distinctive clinical or biochemical features (Chelly et al. 2001). Both MRXS and MRX have been localized to all regions of the X chromosome (Chiurazzi et al. 2001).

We detected a de novo pericentric inversion of the X chromosome (46,X,inv[X][p21.3q27.1]) in a female pa-

tient with mild MRX. She was the second child born to healthy, unrelated parents. Her elder sister was normal, and there was no significant family history. She was born at 41 wk of gestation after a normal pregnancy and delivery. Birth weight was 3,380 g (25th–50th percentile), length 49 cm (25th percentile), and head circumference 35 cm (>50th centile). When the patient was 6 mo old, her mother was concerned about significant hypotonia. Developmental milestones were delayed in all areas. The child did not sit unsupported until 1 year of age and walked at 3 years of age. Language development was delayed, and she did not speak until the age of 3 years 6 mo. She had strabismus and was operated on at 5 years of age. Clinical examination at age 10 years 9 mo showed no physical abnormalities. No facial dysmorphism was noted. Speech was low and hypernasal. There were no behavioral difficulties. Cognitive assessment showed mild mental retardation (IQ 50–55) characterized by a memory deficit, moderate language delay with a low vocabulary, and poor reasoning.

Received July 24, 2002; accepted for publication September 4, 2002; electronically published November 8, 2002.

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To fine map the breakpoints, we performed FISH analyses using YAC, BAC, or PAC clones, obtained from the CEPH YAC library and from the CHORI center, as probes on metaphase chromosomes from the patient. After hybridization and washing, we detected biotinylated probes using antibiotin antibodies coupled to fluorescein isothiocyanate and digoxigenin probes using antidigoxigenin antibodies coupled to rhodamin. Chromosomes were counterstained with DAPI/antifade. The slides were observed under a Zeiss epifluorescence microscope (Axio-phot) connected to the Powergene 810 probe system (Perspective Scientific International). The locations of the inversion breakpoints and contigs spanning the breakpoints are shown in figure 1, as is localization of genes near the Xp and Xq regions.

FISH analyses showed that the YAC clone 453B11 extended over the Xp and Xq breakpoints (fig. 1a and 1b) and that the Xq breakpoint was located in an area

of 100 kb delimited by the BAC clones RP11-51C14 and RP11-189F12 (fig. 1a). Sequence data of the clones surrounding the overlapping regions indicated the presence of the *IL1RAPL* (IL-1 receptor accessory protein like) gene (Carrié et al. 1999) in the Xp breakpoint. We performed Southern blot analyses using the full-length cDNA of *IL1RAPL* (kindly provided by A. Carrié), and no abnormal restriction profile was detected in the female patient (data not shown). Polymerase chain reactions (PCR) of the *IL1RAPL* exons revealed that the YAC clone 453B11 contained only the sixth exon of the gene. The *IL1RAPL* gene, composed of 11 exons and involved in MRX, was disrupted in an intronic region near the sixth exon.

However, we could not exclude the possibility that an additional gene, located at the Xq breakpoint, was involved in the mental retardation (MR) of this female patient, because (i) there is evidence of a lack of mental

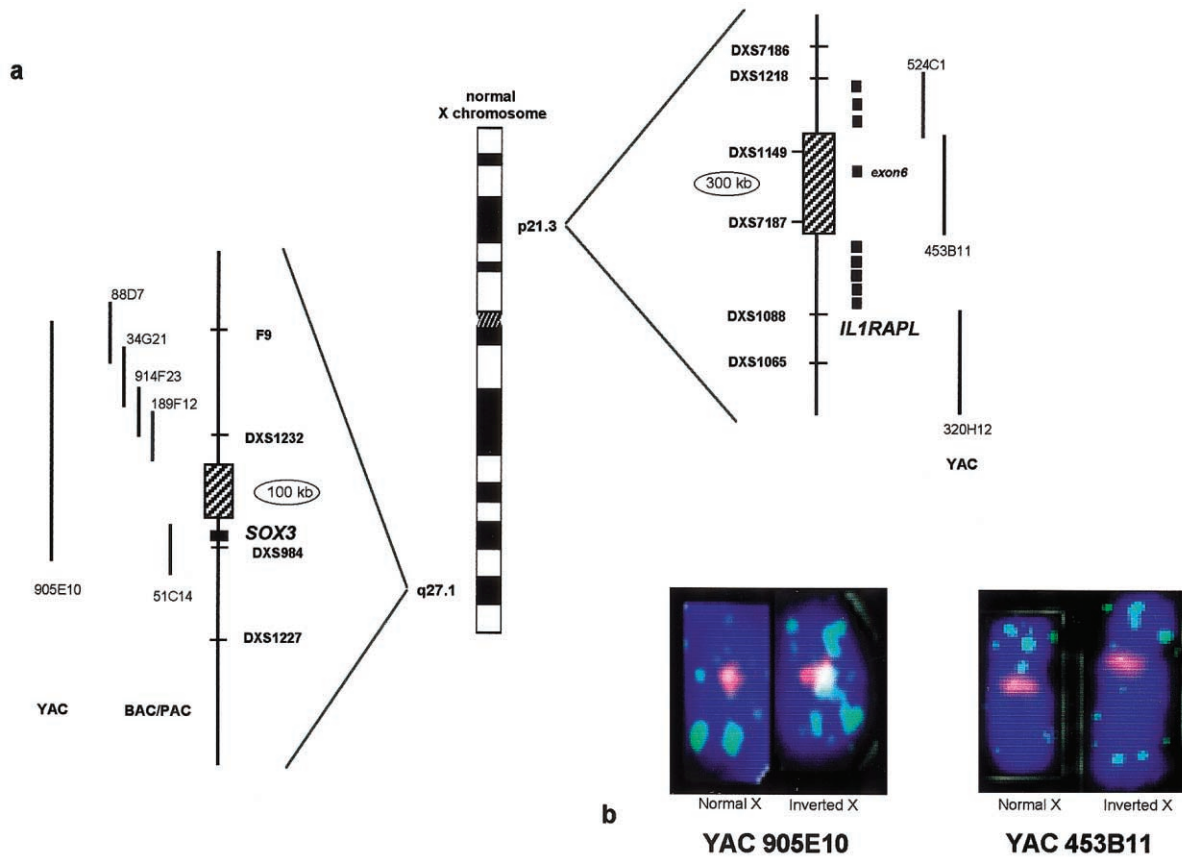
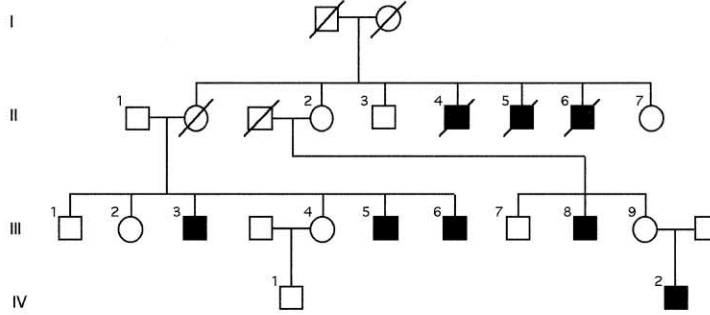


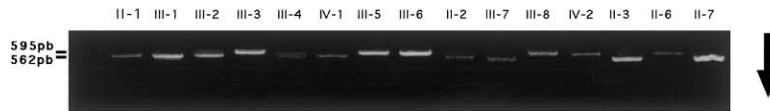
Figure 1 Physical map of the breakpoints of the pericentric inversion of the X chromosome. *a*, Physical map of the pericentric inversion 46,X,inv(X)(p21.3q27.1). The positions of the two breakpoint areas are indicated by diagonally striped boxes. The markers and STS are indicated (*dashes*). Their mapping showed that the *IL1RAPL* gene was disrupted by the Xp breakpoint near the 6th exon. Moreover, the Xq breakpoint area (100 kb) was localized 100 kb centromeric from the *SOX3* gene. *b*, Results of in situ hybridization analyses on metaphases of the patient with pericentric inversion, showing FISH signals obtained on normal and inverted X chromosomes with YAC clones 453B11 and 905E10 (*green*) for the Xp and Xq breakpoints, respectively, and with a chromosome-X- α phasatellite probe (*red*) (Qbiogen). Note the hybridization signals on the inverted X chromosome on both the Xp and Xq sides, indicating that the two YAC clones spanned the breakpoints.

A

(a)



(b)



normal allele

nt 694
 ccc ggt gcc gcg gcc gcc gcc gcc gct gcc gcg gcc gca gcc gct gcc gcc agc agt
 P G A A A A A A A A A A A A A A S S
 AA 232

(c)

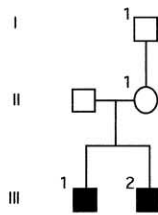
mutated allele

Duplication of 33 bp
gcc gcc gct gcc gcg gcc gca gcc gct gcc gcc
 A A A A A A A A A A A A

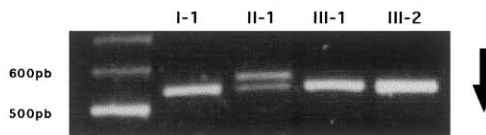
nt 694
 ccc ggt gcc gcg gcc gcc gcc gcc gct gcc gcg gcc gca gcc gct gcc gcc agc agt
 P G A A A A A A A A A A A A A A S S
 AA 232

B

(a)



(b)



normal allele

nt 694
 ccc ggt gcc gcg gcc gcc gcc gcc gct gcc gcg gcc gca gcc gct gcc gcc agc agt
 P G A A A A A A A A A A A A A A S S
 AA 232

mutated allele

nt 694
 ccc ggt gcc gcg gcc gcc gcc gcc agc agt
 P G A A A A A A S S
 AA 232

impairment in carrier females with a microdeletion in the *IL1RAPL* gene (des Portes et al. 1998; Carrié et al. 1999), (ii) a male patient has been reported with a 1.5-Mbp deletion of the *F9-DXS984* region (that overlaps our Xq breakpoint) associated with hemophilia and mental retardation (Rousseau et al. 1991), and (iii) the *SOX3* gene (SRY [sex determining region Y]-box 3 [MIM 313430]) that encodes for a transcription factor that is highly expressed in the fetal brain (and not in adult brain) and involved in neural development (Stevanovic et al. 1993; Collignon et al. 1996) was localized at 100 kb from the Xq breakpoint. No other genes have been identified in the breakpoint area, according to the Ensembl database. The *SOX3* gene can be submitted to position-effect phenomena (Kleinjan et al. 1998) caused by inversion of the X chromosome. Indeed, translocation breakpoints scattered over 1 Mbp of the *SOX9* gene have been reported to cause campomelic dysplasia (Pfeifer et al. 1999), caused by the removal of regulatory elements of the expression of *SOX9*. Abnormalities of the regulatory regions have also been described for the *SRY* gene (McElreavey et al. 1992, 1996), which probably originates from the *SOX3* gene (Collignon et al. 1996). We postulated that inversion could lead to dysregulation of the expression of the *SOX3* gene.

To investigate the effect of the inversion on the expression of the *SOX3* gene, we performed RT-PCR on RNA extracted from lymphoblastoid cell lines (LCL) of the patient. We were unable to detect *SOX3* transcripts in either LCL from the patient or in LCL from normal controls, thus demonstrating that this gene was not expressed in LCL. Analysis of X chromosome inactivation on LCL from the patient, at the AR locus (Xq13), revealed a random inactivation profile (data not shown), confirming that the gene involved in the patient's MR was not expressed in LCL.

The pericentric inversion of the X chromosome in this female patient physically disrupted the *IL1RAPL* gene. However, *SOX3* could not be excluded, although we could not test its expression.

Just as homeodomain genes are known to play crucial roles in the establishment of cerebral structure, *SOX3* has been considered a strong candidate gene for other

cases of XLMR. We screened for mutations in all available families with MRX overlapping the Xq26-q27 region collected by the European XLMR consortium (15 unrelated mentally retarded males including two with XLMR and growth hormone deficiency [GHD] [T19 and N3]) and two Australian families with XLMR and GHD (one described by Zipf [1977]) by direct sequencing of PCR products corresponding to the entire coding region of the *SOX3* gene (GenBank accession number NM_005634).

Families N3 and T19 have already been described clinically by Hamel et al. (1996), Hol et al. (2000) (N3), and Raynaud et al. (1998) (T19). Briefly, all patients in family N3 were short and mentally retarded. Clinical examination demonstrated facial anomalies in some but not all of the patients. On the average, untreated patients reached their final height, ranging from 135–159 cm, at the age of 24–25 years. In all patients examined, behavior was considered infantile, and laboratory investigations demonstrated total GHD. In family T19, the disease was characterized in males by mental retardation, short stature, microcephaly, and peculiar face. Mental retardation was mild to borderline. Height and head circumference were <-2.2 SD in all affected males and females. Facial anomalies, consisting of general "coarseness," highly curved forehead and midface hypoplasia, and concave nasal bridge with nasal tip of normal size, were observed in affected males and females.

Abnormalities were found in two patients. One in-frame duplication of 33 bp (711–743 dup) encoding for an additional 11-alanine tract was found in family N3, with XLMR and isolated GHD (fig. 2A). This duplication is predicted to cause expansion of the normal polyalanine tract (amino acids 234–249) by 15 to 26 alanine residues (figs. 2A and 3). Cosegregation of this duplication with the disease was confirmed in this large family (fig. 2A). This mutation was not found in 600 normal X chromosomes or in 197 unrelated FRAXA-negative mentally retarded patients, and no polymorphism in the tract was detected in controls. Furthermore, one deletion of 27 bp (718del27), consisting of a deletion of nine alanine residues in the same polyalanine tract,

Figure 2 Expansion of polyalanine tract detected in the family with mental retardation and GHD (N3) and deletion in the same polyalanine tract in family T84. *A*, Family N3. *a*, Family pedigree. *b*, Two percent agarose gel electrophoresis of PCR products corresponding to amplification of part of *SOX3* containing the 33-bp duplication. PCR amplification of *SOX3* was performed using 50–100 ng of genomic DNA, 0.5 μ M primers described elsewhere by Xiang et al. (2000), and 2.5 U of *Taq* polymerase (Promega). The PCR cycles consisted of an initial denaturation step of 95°C for 5 min, followed by 30 cycles of amplification (95°C for 1 min, 60°C for 1 min, 72°C for 1 min) and a final cycle at 72°C for 7 min. Fifteen family members, including six affected males, were available for this study who showed cosegregation of the duplication with the phenotype. *c*, The resulting amino acid sequence of 711–743dup (33 bp) is indicated in italics. The PCR products were purified by gel electrophoresis and QIAquick gel extraction kit (QIAGEN) and sequenced by ABI BigDye terminator cycle sequencing chemistry on an ABI377 automated sequencer. *B*, Family T84. *a*, Family pedigree. *b*, Two percent agarose gel electrophoresis of PCR products corresponding to amplification of part of *SOX3* containing the 27-bp deletion. The deletion was observed for the two affected males, their healthy mother, and their maternal grandfather. *c*, The resulting amino acid sequence of 718del27 (27 bp) is indicated in italics.

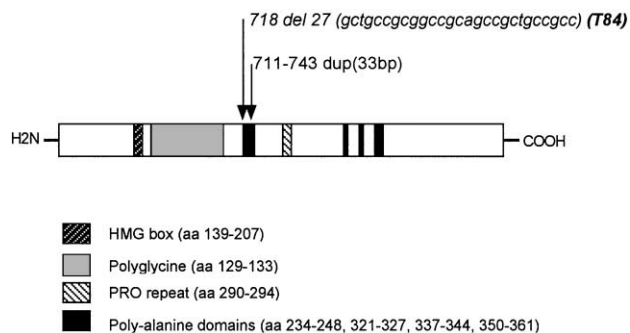


Figure 3 Position in the *SOX3* gene of the mutations identified in the MR family with GHD and in family T84. The polymorphic deletion is indicated in italics.

was observed in two severely mentally retarded boys of family T84 (figs. 2B and 3). However, it is likely that this sequence variation corresponded to a rare polymorphism because of the detection of this deletion in the healthy maternal grandfather.

No abnormalities were found in the other families with MR and GHD (T19 and the two Australian families) in the coding region of *SOX3*.

Mutations in homeobox genes have been shown to play a role in the pathogenesis of numerous conditions in man. Furthermore, seven genes have been described in which alanine expansions have been shown to cause human disease: *FOXL2* (Crisponi et al. 2001), *HOXA13* (Goodman et al. 2000), *HOXD13* (Muragaki et al. 1996), *PABP2* (Brais et al. 1998), *RUNX2* (Mundlos et al. 1997), *ZIC2* (Brown et al. 2001), and, recently, *ARX* (Bienvenu et al. 2002; Stromme et al. 2002), which is associated with specific and nonspecific XLMR. It is interesting that the authors also found both a polyalanine duplication and a rare polymorphism in the *ARX* gene due to a deletion. The function of such extended polyalanine tracts is not yet known, but it has been suggested that they suppress transcriptional activity of these proteins (Han et al. 1993).

SOX3 is a member of the SRY-related HMG box family and acts as a transcription factor gene of unknown function which is highly expressed in the human fetal brain and spinal cord. This gene is a homolog of the murine *Sox3* gene, which is expressed at high levels in neuronal tissue during development and is thought to play a role in neuronal development (Stevanovic et al. 1993; Collignon et al. 1996). Expression analysis using mouse models has also revealed that *Sox3* is expressed in the developing pituitary gland (Collignon et al. 1996). Furthermore, functional studies of *Sox3* in fish embryos have shown that increased levels of this gene in the developing lens and otic placodes cause severe hypoplasia of these tissues (Koster et al. 2000). This could explain

the phenotype in the affected patients in the family with the duplication. Solomon et al. (2002) recently showed increased gene levels at Xq26-27 associated with X-linked hypopituitarism and variable degrees of mental retardation due to large duplicated regions in the Xq26.1-q27.3 region, including the *SOX3* gene. Moreover, they postulated that *SOX3* might be involved in hypopituitarism due to overexpression of this gene.

SOX3 is a new gene which we have identified as mutated in XLMR associated with GHD. However, the absence of mutation of this gene particularly in family T19 suggests genetic heterogeneity of this condition.

Acknowledgments

We thank the patients, family members, and the European XLMR Consortium for their participation in this study and Brigitte Jauffrion for technical assistance. This work was supported by grants from Institut National de la Santé et de la Recherche Médicale (INSERM) and Fondation Jerome Lejeune.

Electronic-Database Information

Accession numbers and URLs for data presented herein are as follows:

Ensembl database, <http://www.ensembl.org/>
 GenBank, <http://www.ncbi.nlm.nih.gov/Genbank/> (for *SOX3* mRNA [accession number NM_005634])
 Online Mendelian Inheritance in Man (OMIM), <http://www.ncbi.nlm.nih.gov/OMIM/> (for *SOX3* [MIM 313430])

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