Short reports

Interstitial deletion in Xp22.3 is associated with X linked ichthyosis, mental retardation, and epilepsy

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Abstract

We describe monozygotic male twins with an interstitial deletion of Xp22.3 including the steroid sulphatase gene (STS). The twins had X linked ichthyosis, X linked mental retardation, and epilepsy. A locus for X linked mental retardation has been assigned to a region between STS and DXS31 spanning approximately 3 Mb. Recently the locus was further refined to an approximately 1 Mb region between DXS1060 and GS1. By PCR analysis of flanking STS gene markers in our patients we succeeded in narrowing down the locus to between DXS6837 and GS1.

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Interstitial and terminal deletions of Xp22.3 in males are associated with monogenic disorders such as short stature, chondrodysplasia punctata, mental retardation, X linked ichthyosis, Kallmann syndrome, and ocular albinism. Depending on the length of the deletion, these disorders occur independently from each other or in combination as a contiguous gene syndrome.^{1,2} X linked ichthyosis (XLI) is caused by mutations in the steroid sulphatase (STS) gene with an incidence of 1:2000 to 1:6000 males.^{3,4} X linked mental retardation (XMR) has an incidence of about 1:600 male births. Several loci on the X chromosome are thought to be associated with mental retardation.⁵ One of these loci was localised to Xp22.3 between DXS1060 and GS1.⁶

We report on monozygotic male twins with X linked ichthyosis, mental retardation, epilepsy, and an interstitial deletion at Xp22.3 involving STS. The extent of the Xp deletion was analysed by examining flanking STS gene markers.

Methods

STS DELETION

DNA was prepared from fresh or frozen venous blood samples by the salting out procedure.⁷ Restriction enzyme digestion with *TaqI* and *EcoRI* and Southern blotting were performed by standard techniques. A full length cDNA of the steroid sulphatase gene (probe 5'3-STS,

kindly provided by Dr von Figura, Göttingen) was used as hybridisation probe.

SPECIFIC MARKERS

Further Xp22.3 specific markers, *PABX*, DXS1060, DXS996, DXS1139, DXS278, and *KAL*, were tested by the polymerase chain reaction as previously described.^{2,8} PCR primers for DXS6837 and DXS6834 were newly established in this study.⁹ DXS6837: forward primer 5'-ATT CAT CAT ATA TAC ATC AG, reverse primer 5'-AGT CAT ACT TTA ATC TTG C, annealing temperature 62°C, product size 88 bp. DXS6834: forward primer 5'-CCA ATC AGA GAG CTC AG, reverse primer 5'-ATC TGC TCA TGC TTC TC, annealing temperature 57°C, product size 148 bp.

Case report

Patients 1 and 2 were male monozygotic twins who were born at term in 1987 after an uneventful pregnancy and caesarian section at term in 1987. Birth weights were 2525 g and 2870 g, lengths 48 and 50 cm, and head circumferences 32 and 33 cm. Apgar scores were 10/10/10 for both. Both had ichthyosis from birth and both had grand mal seizures from their first year of life onwards. Both had unilateral cryptorchidism and were operated on at the age of 2 years. At the age of 12 months psychomotor development was significantly retarded. At the age of 8 years, intelligence was tested (Kaufman-Assessment Battery for Children) and showed similar decreased intellectual abilities (IQs of 60 and 70, respectively). Height was normal (132 cm, 25th centile). MRI of the brain was normal in both patients.

There were two other male family members affected. One maternal uncle was reported to have epilepsy, ichthyosis, and mild mental retardation. He refused to be examined or tested. A three year old brother had ichthyosis but no epilepsy. His psychomotor development screened by the "Griffith test" was slightly retarded (developmental quotient 73, normal range >80). Three other sibs, two girls and one boy, were not affected and showed normal psychomotor development.

Results

In both patients, a complete deletion of the STS gene was observed by Southern analysis. Only bands characteristic for the Y specific

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		← Telomere				Centromere →					
		Locus									
Patient		PABX	DXS1060	DXS996	DXS6837	DXS6834	DXS1139	STS	DXS278	KAL	
1		+	+	+	+	-	-	-	+	+	
2		+	+	+	+	-	-	-	+	+	

Figure 1 Deletion map of patients 1 and 2. - = deleted, + = present. The locus order is based on the reports of Schaefer et al,² Franco et al,¹⁰ and Herrell et al.⁸

STS pseudogene were observed upon hybridisation with the cDNA probe 5'3 STS. The genomic interval between PABX and KAL was investigated with PCR analysis. PABX, DXS1060, DXS996, DXS6837, DXS278, and KAL were present, whereas DXS6834 and DXS1139 were deleted. Thus, the distal breakpoint maps between DXS6837 and DXS6834 and the proximal breakpoint between STS and DXS278 (fig 1).

By analysing the DNA samples of the family, we also found the same deletion in the partially affected brother and no deletion in the unaffected brother (data not shown). Results of the mother showed that she is a carrier, while two sisters are not carriers. The PCR results for DXS6837 and DXS6834 in the family are shown in fig 2.

Figure 2 Results of the PCR analysis of markers DXS6837 and DXS6834. (1: father, 2: mother, 3: patient 1, 4: patient 2, 5: affected brother, 6: sister, 7: sister, 8: unaffected brother, 9: control, M: molecular weight marker).

Discussion

The monozygotic twins reported here have XLI, mental retardation, and epilepsy. Southern and PCR analysis in the patients showed an interstitial deletion with the telomeric breakpoint between DXS6837 and DXS6834 and centromeric breakpoint between STS and DXS278. The deletion was shared by the mother and by another affected brother. Since STS is deleted in our patients this would explain the X linked ichthyosis in this family. In addition, both of our patients and the affected brother are mentally retarded. This indicates that an X linked mental retardation (MRX) gene is deleted or disrupted in our patients. About 65 putative MRX loci have been mapped to the X chromosome so far.¹¹ However, because of overlapping regions only 10 to 12 MRX genes can be considered. By deletion mapping, one locus has been located in the Xp22.3 region between DXS31 and STS, spanning approximately 3 Mb.¹² Muroya et al¹³ analysed an interstitial deletion at Xp22.3 in a patient with mental retardation. The results of the deletion mapping in the patient were compared with the Xp22.3 deletion breakpoints of three patients with loss of STS, KAL1, and OAI but without mental retardation described by Sunohara et al¹⁴ and Schaefer et al.² Muroya et al¹³ further refined the MRX locus to an approximately 1.5 Mb region between DXS1060 and DXS1139. Recently, Weissörtel et al⁶ located the MRX gene between DXS1060 and GS1 spanning a roughly 1 Mb region according to the X Chromosome Workshop consensus map. By deletion analysis in our patients, we succeeded in further narrowing down the locus of the putative MRX gene. The telomeric breakpoint of the interstitial deletion in our patients lies proximally to DXS6837. Therefore, we propose that the MRX gene is present in the less than 1.5 Mb region between DXS6837 and DXS1139. Epilepsy is an additional manifestation of our patients. However, another affected brother with the deletion in Xp22.3 does not have seizures. Spranger et al¹⁵ reported a men-

tally retarded patient with a terminal Xp22.3 deletion and myoclonic epilepsy. The breakpoint of the deletion was distal to *GS1*. DXS6837 and DXS6834 were not tested in this study. Since the patient described by Spranger *et al*⁵ had mental retardation, the putative *MRX* locus may be included in the deletion. Therefore, in this patient the distal part of the deletion present in our patients may also be deleted. Further studies are needed to determine if a gene predisposing to epilepsy is located between *GS1* and DXS6837.

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