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ORIGINAL INVESTIGATION

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Xp deletions associated with autism in three females

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Abstract We report eight females with small deletions of the short arm of the X chromosome, three of whom showed features of autism. Our results suggest that there may be a critical region for autism in females with Xp deletions between the pseudoautosomal boundary and *DXS7103*. We hypothesise that this effect might be due either to the loss of function of a specific gene within the deleted region or to functional nullisomy resulting from X inactivation of the normal X chromosome.

Introduction

The distal region of Xp shows a high frequency of chromosomal rearrangements, which have led to the identification and mapping of several disease genes (Ballabio and Andria 1992). By correlating the clinical phenotypes and genotypes of patients with either deletions or translocations involving Xp, we have identified a possible critical region for autism, proximal to the pseudoautosomal boundary and distal to the marker *DXS7103*.

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Materials and methods

Patients

The study population comprised eight patients, all with deletions or (X;Y) translocations involving Xp22 and distal to or overlapping the putative microphthalmia with linear skin defects syndrome (MLS) locus (Wapenaar et al. 1993; Cox et al. 1998). The clinical phenotypes of Cases 2, 3, 4, 6, 7, and 8 have been described in an earlier study (James et al. 1998) in which they were, respectively, Cases 1, 2, 3, 4, 5 and 6. Case 8 was one of the earliest patients described with MLS, and is Case 2 in Al-Gazali et al. (1990). The present report includes all the females studied by us with a deletion of Xp distal to *DXS1224*. Case 4 was ascertained because she is the mother of Case 3; the other six patients were all unrelated and ascertained for a variety of reasons (Table 1).

Cytogenetic analysis

Translocation and deletion breakpoints were determined by analysis of G-banded metaphase chromosomes harvested from peripheral blood lymphocytes. An additional culture was established for some patients to determine the replication timing of the sex chromosomes by pulse-labelling with 5-bromo-2-deoxyuridine (BrdU) (minimum of 30 cells scored). Karyotypes were also determined for the mother of Case 1 and both parents of Cases 2, 3, 5, 6, 7, and 8.

Fluorescence in situ hybridization (FISH) analysis

The FISH studies were carried out using the standard method described by Pinkel et al. (1986). Hybridization signals from biotin or digoxigenin (DIG)-labelled probes were detected using either single layers of avidin-fluorescein isothiocyanate for biotin or anti-DIG-tetramethylrhodamine isothiocyanate and analysed using a conventional Zeiss epi-fluorescent Axiophot microscope. Images were captured by a cooled CCD camera (Photometrics), and enhanced and analysed using Digital Scientific (Vysis) Smart Capture software.

Molecular analysis

DNA was extracted from peripheral blood and buccal scrapes by a salt precipitation technique (Miller et al. 1988). The parental origin of the normal X chromosome was determined by polymerase chain reaction (PCR) amplification of microsatellite markers located within the region deleted in the structurally abnormal chromosome. Similarly, breakpoints of the deletions and translocations were mapped by PCR

Table 1 Details of the eight female patients. (*MLS* microphthalmia with linear skin defects syndrome)

Case number	Patient ID	Karyotype	Ascertainment	Origin of the deleted X chromosome	
1	97-05436	46,X,del(X)(p22.32)	Short stature	Paternal	? De novo
2	83-04399	46,X,t(X;Y)(p22.31;q11.21)	Short stature	Paternal	De novo
3	95-02697	46,X,t(X;Y)(p22.33;q12)	Turner syndrome	Maternal	Familial
4	95-D1130	46,X,t(X;Y)(p22.33;q12)	Mother of Case 3	Paternal	? De novo
5	97-05314	46,X,del(X)(p22.13–p22.32)	Autism	Paternal	De novo
6	96-01693	46,X,del(X)(p22.31 or p22.32)	Developmental delay	Paternal	De novo
7	95-02669	46,X,del(X)(p22.31)	Turner syndrome	Paternal	De novo
8	86-01593	46,X,der(X)t(X;Y)(p22.3;q11.2)	MLS	Paternal	De novo

amplification using a panel of markers spanning distal Xp to define regions of hemizygosity. The relative order of the panel of markers was derived from the breakpoint mapping by integrating maps from GDB and Genethon (Dib et al. 1996). For Cases 3 and 4, markers on proximal Xp and proximal Xq were also used to investigate possible recombination between the normal and deleted maternal X chromosomes.

Standard PCR conditions were used throughout and all primer sequences are available on GDB. A fluorescent or radioactive label was incorporated into one primer from each pair, so that PCR products could be visualized by autoradiography following electrophoretic separation on a denaturing 6% polyacrylamide gel, or analysed on an ABI 377 automated DNA sequencer (Perkin Elmer).

Determination of X-inactivation pattern

The X-inactivation status of the *HUMAR* locus on Xq13 was determined for all females in the study, using a fluorescent PCR assay described by Allen et al. (1992) and modified by Pegoraro et al. (1994). The extent of methylation-sensitive enzyme cleavage of each allele can be quantified and is proportional to the degree of methylation, or inactivity, at that locus on each X chromosome. Whenever possible the X-inactivation pattern was determined on DNA from both peripheral blood and buccal smears. Control male DNA was used to ensure complete enzymatic cleavage of unmethylated alleles.

Results

Clinical data

Cases 2, 3, 4, and 7 have variable phenotypes that are described in detail in James et al. (1998). All cases except for Case 5 were of short stature, presumably because of haploinsufficiency for the *SHOX* gene located in the pseudoautosomal region (Rao et al. 1997). Case 1 was short with no other mental, physical or behavioural impairments. Of the eight patients, Cases 5, 6 and 8 were diagnosed as having autism, and are described in more detail below. There was no evidence for autism in the other five patients.

Case 5 had no external physical abnormalities and her height, weight and head circumference were normal. Her development gave cause for concern at about 1 year of age, and a diagnosis of autism was suggested when she was 3 years old. An autism diagnostic interview (ADI) carried out at 10 years of age confirmed this diagnosis. She showed qualitative impairments in reciprocal social interaction and communication, and stereotyped interests and patterns of behaviour.

Case 6, as noted by James et al. (1998), had short stature, increased inner canthal distance (3.4 cm), upward-slanting palpebral fissures, an alternating convergent squint, projecting ears with bilateral prehelical pits, and middle ear effusions requiring grommets. She walked at 19 months and was thought to have severe learning disabilities and probable autism. An ADI carried out at 2.5 years of age confirmed the diagnosis of autism, showing qualitative impairments in reciprocal social interaction, verbal and non-verbal communication, and repetitive and stereotyped patterns of behaviour.

Case 8 is a patient with MLS syndrome and was reported as Case 2 by Al-Gazali et al. (1990). Her intelligence is normal. She showed a precocious use of language, plus an unusual degree of musical ability, from early childhood. Concerns about her behaviour have been expressed in the last few years. An ADI was carried out at the age of 12 years. Some items were difficult or impossible to evaluate because of the subject's blindness. It was concluded that she could be described as a child with high-functioning autism, fulfilling criteria for autism on qualitative impairments in social interaction, impaired verbal communication, and repetitive behaviours and stereotyped patterns of behaviour. She did not score above criteria for autism on non-verbal communication, where her visual impairment was felt to account for her lack of abnormal behaviour.

Cytogenetic analysis

The karyotypes are shown in Table 1 and were apparently non-mosaic for all eight females. Seven patients had terminal Xp deletions, although in four cases this was caused by a translocation between the short arm of the X chromosome and the long arm of the Y chromosome. One patient (Case 5) had an interstitial deletion. The very small deletions in Cases 1, 2 and 5 were confirmed by FISH and molecular analysis. Cases 4 and 3 (mother and daughter) had identical structural rearrangements and this was verified by molecular analysis.

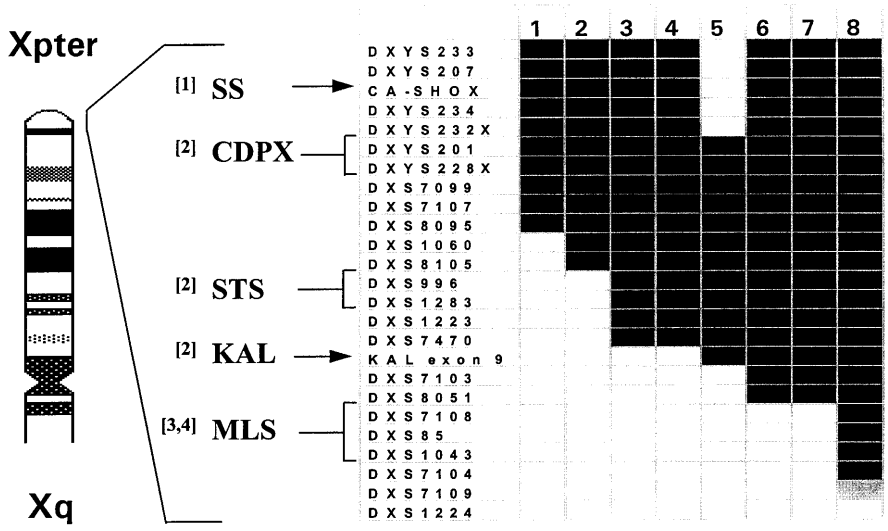

Fig. 1 Molecular mapping of Xp22 breakpoints. The extent of each patient's deletion is indicated by *black shading* while the *unshaded* area represents the extent of heterozygosity. *Grey shading* represents a non-informative marker. *[1]* Rao et al (1997), *[2]* Ferrero et al . (1995), *[3]* Wapenaar et al. (1993), *[4]* Cox et al. (1998). (*SS* SHOX stature gene; *CDPX* X-linked chondroplasia punctata; *STS* steroid sulphatase deficiency; *KAL* X-linked Kallmann syndrome; *MLS* microphthalmia with linear skin defects)

Molecular analysis

Origin of the deleted X chromosome

Parental DNA was available from both parents for six patients and from the mother for Cases 1 and 4. In all cases biparental inheritance of the X chromosome was observed, excluding uniparental disomy. Given that the fathers of Cases 1 and 4 were described as being phenotypically normal, the deletion in these families is likely to have arisen de novo. One case was familial, with mother-to-daughter transmission of the structurally abnormal X, while all deletions/translocations confirmed or assumed to have arisen de novo were of paternal origin. This is consistent with the observation that the great majority of X-chromosome deletions involve the paternal X chromosome and presumably occur during male gametogenesis (James et al. 1998).

Mapping of breakpoints

The results of the deletion mapping for all eight females are shown in Fig. 1. Several disease-causing genes have been mapped to Xp22 (Ballabio and Andria 1992) and the approximate locations of five of these are marked on Fig. 1.

X inactivation

The X-inactivation results in Table 2 demonstrate good agreement between the cytogenetic and molecular methods and between the values determined from whole blood (mesoderm) and from buccal cells (ectoderm). The one discordant result was found for Case 8. At the *HUMAR* locus the abnormal X was found to be active in a small proportion of cells from all tissues studied. This is consistent with the hypothesis that the phenotype in MLS syndrome is produced by a structurally abnormal X remaining active in a sufficient proportion of cells (Lindsay et al. 1994). However, in all cells investigated by BrdU labelling (n=150), the abnormal X was always late replicating. While the molecular analysis is performed on total DNA extracted from all white cells, the cytogenetic result is derived from only a subset of white cells, T lymphocytes.

Table 2 X-inactivation results (% cells in which the structurally abnormal X is active)

Case number	Cytogenetic Lymphocytes	Molecular analysis		
		White cells	Mouth brush	Urine
1	–	64	–	–
2	58	69	–	–
3	33	34	13	–
4	12	5	3	–
5	–	90	–	–
6	60	78	94	–
7	70	88	92	–
8	0	19	12	10

In the other seven females tested, the abnormal X was also found to be active in at least a proportion of cells. In contrast, females with deletions proximal to Xp22 have complete skewing in their lymphocytes (Schaefer et al. 1993; James et al. 1998).

The cytogenetic analysis measures replication timing of the whole X chromosome, whereas the molecular analysis measures the extent of methylation only at the *HUMAR* locus. Therefore, in cases of maternal inheritance of a structurally abnormal X chromosome, recombination can occur between the breakpoint of the structural abnormality and the *HUMAR* locus and it is important to determine the origin of the daughter's maternal *HUMAR* allele. Recombination occurred in the interval between the markers *DXS451* and *DXS8025* for Case 4 (data not shown), such that the maternal X chromosome inherited by her daughter, Case 3, is of grandpaternal origin (and carrying the structural abnormality) distal to *DXS451* and of grandmaternal origin

proximal to *DXS8025*. Therefore, while Cases 3 and 4 have the same inherited structural abnormality, the maternal *HUMAR* allele of Case 3 has come from the normal X chromosome of Case 4.

Discussion

Deletions of the Xp22 region produce a range of different clinical phenotypes depending on both the sex of the individual and the extent and location of the deleted chromosomal material (Ballabio and Andria 1992). Thus phenotype/genotype correlations can lead to the identification of novel disease genes.

Autism is a neuropsychiatric disorder characterized by early onset with impaired social, language and cognitive development (Lotspeich and Ciaranello 1993). The incidence of autism in the general population is between 2 and 5 per 10|000. Between 5 and 14% of autistics have an identifiable chromosomal abnormality or other genetic condition (Rutter et al. 1994; Gillberg and Coleman 1996). Genetic factors undoubtedly play a major aetiological role in the remaining idiopathic cases (Bailey et al. 1995). Segregation analysis predicts that autism involves the interaction of several genes (Jorde et al. 1991; Weeks and Lathrop 1995) and six potential candidate regions have now been identified through a whole-genome screen (International Molecular Genetic Study of Autism Consortium 1998). Genes on the X chromosome do not account for a substantial part of the genetic susceptibility to autism (Hallmayer et al. 1996; International Molecular Genetic Study of Autism Consortium 1998). However, the excess of autism in males compared with females, with a 4:1 sex ratio (Lotspeich and Ciaranello 1993), and an increased incidence of autism reported among females with 45,X Turner syndrome (Skuse et al. 1997) suggest that X-linked genes may be important in a subset of cases.

Of eight females studied with deletions or translocations involving chromosome Xp22.3, we report three who have features of autism. Case 5 has autism but no external physical abnormalities and a gene within her small interstitial deletion may be important in the aetiology of autism. The distal boundary of this deletion is between the markers *DXYS201* and *DXYS232X*, just below the pseudoautosomal region, and the proximal boundary is between exon 9 of the gene for X-linked Kallmann syndrome and the marker *DXS7103*. The interval between *DXYS232X* and *DXS7103*, the maximum size of the deletion, is approximately 6 Mb (Nagaraja et al. 1997). The deletions in the other autistic subjects, Cases 6 and 8, encompass the same region as that of Case 5 but are larger terminal deletions with proximal breakpoints below that of Case 5.

This is the first report of an association between autism and a deletion of Xp, although autism has previously been reported in two patients with structural abnormalities involving this region (Fig. 2). The first was a female with a balanced (X;8) translocation, with a breakpoint within the gastrin-releasing peptide receptor gene (*GRPR*) on Xp22.1,

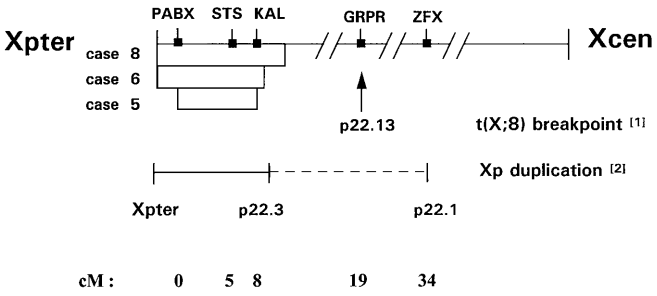

Fig. 2 Map of the Xp22 region highlighting structural abnormalities in autistic cases. *[1]* Ishikawa-Brush et al. (1997), *[2]* Rao et al. (1994). *Open boxes* represent the deletions of Cases 5, 6, and 8. Approximate distances (cM from *PABX*) and marker order taken from Ferrero et al. (1995). [*PABX* pseudoautosomal region boundary, *GRPR* gastrin peptide releasing receptor, *ZFX* zinc finger gene (X linked)]

proximal to the deletion of Case 5 (Ishikawa-Brush et al. 1997). The second was a male infant with a terminal duplication of Xp22, which is known to include the *STS* locus and may extend proximally to the *ZFX* locus (Rao et al. 1994).

The occurrence of three autistic females all with small deletions of Xp22.3 could be due to chance, although this seems unlikely with such a low incidence of autism in the general female population (Lotspeich and Ciaranello 1993). However, Cases 3, 4 and 7 are also monosomic for this region and although they have more complex and variable phenotypes than Case 5, they are not autistic (James et al. 1998). We propose the following three hypotheses to explain why some but not all females monosomic for the region deleted in Case 5 have autism.

Hypothesis 1 proposes that the deletion includes a gene that is normally subject to X inactivation in females. Normal males and females have one functional copy. If the deletion is small enough to be compatible with the structurally abnormal X remaining active, functional nullisomy for this gene can arise in a proportion of cells. This increases the risk of autism, either by a local effect in the brain or by a systemic effect. X-inactivation status influences the penetrance of autism in females carrying a deletion. Loss of the gene would not account for any increased risk of autism in females with a 45,X constitution, because of obligatory activation of the normal X chromosome in these females. Furthermore, differences in the normal level of expression could not explain the male excess of autism, as males and females would be functionally equivalent.

Hypothesis 2 predicts that the deletion includes a gene that is not subject to inactivation in females. Normal females have two functional copies, while normal males may have one or two functional copies depending on whether there is a Y homologue. Haploinsufficiency in females increases the risk of autism, and is independent of X-inactivation status. Females with large deletions of Xp or a 45,X constitution would be at an increased risk of autism as a result of deletion of this gene. If normal males had one functional copy of the gene, females in whom the gene was deleted would be functionally equivalent to normal males with respect to the development of autism.

In both hypotheses 1 and 2, autistic males with deletions and mutations of the gene should exist, although autism could be obscured by other clinical abnormalities, especially in the case of deletions. X-linked pedigrees of autism would be expected; however, such pedigrees have not been demonstrated.

Hypothesis 3 proposes that autism can arise as a non-specific effect of mosaicism for a near-lethal cell line in the brain. Near-lethality could arise as a result of functional nullisomy for X-linked genes normally expressed in one dose (i.e. inactivated in females), and mosaicism, secondary to X-inactivation pattern, could result if the deletion was not large enough to cause stringent selection against the structurally abnormal X. This mechanism has been suggested for the MLS syndrome (Lindsay et al. 1994), and is supported by the markedly skewed inactivation patterns in Cases 5 and 6, with the structurally abnormal X active in the majority of cells tested. Autism has not previously been reported as a component of MLS, and it would be worthwhile to check for it in other MLS cases. The critical regions for MLS and autism would not necessarily have to overlap, since different tissues might vary in the effect on X inactivation produced by a given deletion. Indeed the critical region for MLS – proximal to the marker *DXS8051* and distal to the marker *DXS1043* – is proximal to the interstitial deletion of Case 5. One might predict for example that a smaller deletion would suffice to ensure obligatory activity of the normal X in brain than in skin. It follows from this hypothesis that the critical region should be difficult to localise, as the effect could depend on deletion of a number of genes having less-specific effects rather than deletion of one critical gene; hence both the size and position of the deletion could be important. While size and position are interdependent for terminal deletions, for interstitial ones they are not. An equivalent condition in males would not be expected under this hypothesis.

This hypothesis is similar to that proposed by Hatchwell (1996) to account for mental retardation and hypomelanosis of Ito in some females with X-autosome translocations. In these cases, there may be a departure from the usual pattern of X inactivation, which is heavily skewed towards inactivation of the normal X. When such skewing does not occur, there will be a significant proportion of cells with functional disomy of genes that are normally subject to X inactivation. Hatchwell et al. (1996) found evidence for this by studying uncultured fibroblasts in one case.

Functional disomy or nullisomy produced by unusual patterns of X inactivation in the presence of a structural abnormality of the X chromosome may be viewed as one mechanism leading to mosaicism for a deleterious cell line. In a wider context, hypomelanosis of Ito has been shown to result from mosaicism for a variety of chromosomal abnormalities (Donnai 1995).

There is an association between hypomelanosis of Ito and autism (Zapella 1992), which suggests that some cases of autism may result from a similar non-specific mechanism of mosaicism within the brain. In the autistic patient of Bolton et al. (1995) with an X;8 translocation, the normal X was late replicating in 20/20 cultured cells, but we have found it to be active in approximately 20% of cells from uncultured skin (data not shown), giving some support to this hypothesis.

Our studies suggest that detailed psychological and developmental assessments should be carried out in females with distal Xp deletions who have been ascertained in infancy or early childhood to determine the true prevalence of autism in this group of patients.

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