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website *extra*

The supplementary data (figs 2–7) can be found on the JMG website

www.jmedgenet.com

Absence of learning difficulties in a hyperactive boy with a terminal Xp deletion encompassing the *MRX49* locus

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EDITOR—The genetic counselling of a pregnant woman who carries an Xp chromosomal deletion is far from straightforward. While the precise locations of the *CDPX1* (arylsulphatase E), steroid sulphatase (*STS*), and Kallman (*KAL1*) genes are known and FISH probes are available for these well characterised genes, the positions of putative mental retardation genes in this region have not yet been determined. Clinical and molecular studies undertaken over

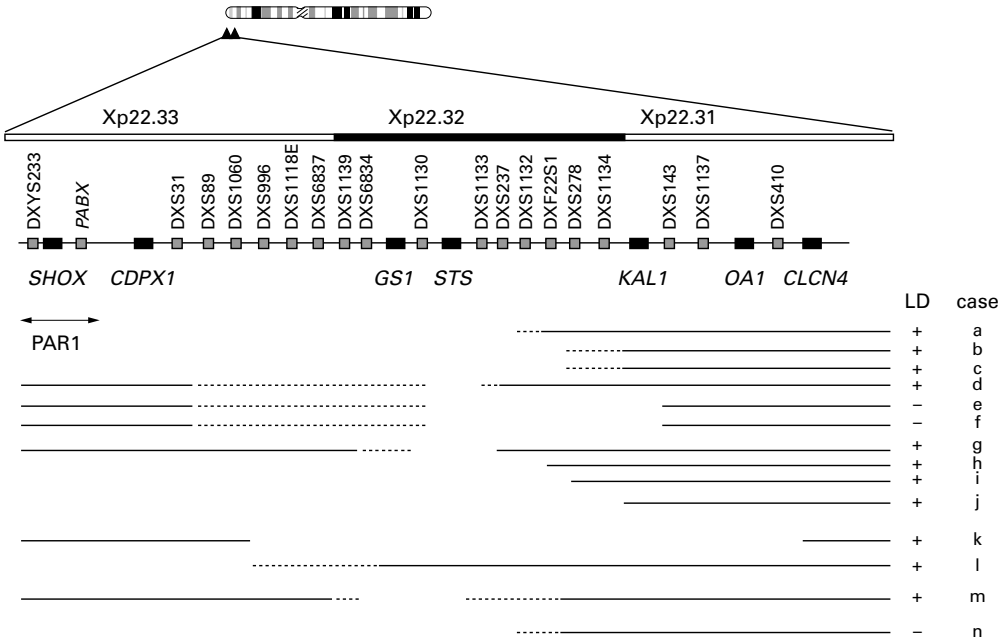

Figure 1 (a) Case 6, (b) case 8, (c) case 9, (d) case 4, (e) case 12, and (f) case 13 of Ballabio et al,[1] (g)-(j) cases BA16, BA20, BA139, and BA75 of Schaefer et al,[3] (k) boy with IQ of 46, short stature, generalised ichthyosis, hypogonadotrophic hypogonadism, nystagmus, and photophobia,[2] (l) boy with aggressive and hyperactive behaviour, myoclonic epilepsy, developmental delay, and no speech aged 4 years 8 months,[4] (m) monozygous male twins with X linked ichthyosis, learning difficulties (LD), and epilepsy,[10] (n) our patient, with short stature, Binder syndrome, and ichthyosis (consistent with the loss of the SHOX, CDPX1, and STS genes, respectively) but no significant learning difficulties. The presence (+) or absence (-) of LD is indicated for each case. A broken line indicates the chromosomal region within which the breakpoint is assumed to lie, while a solid line indicates a retained region.

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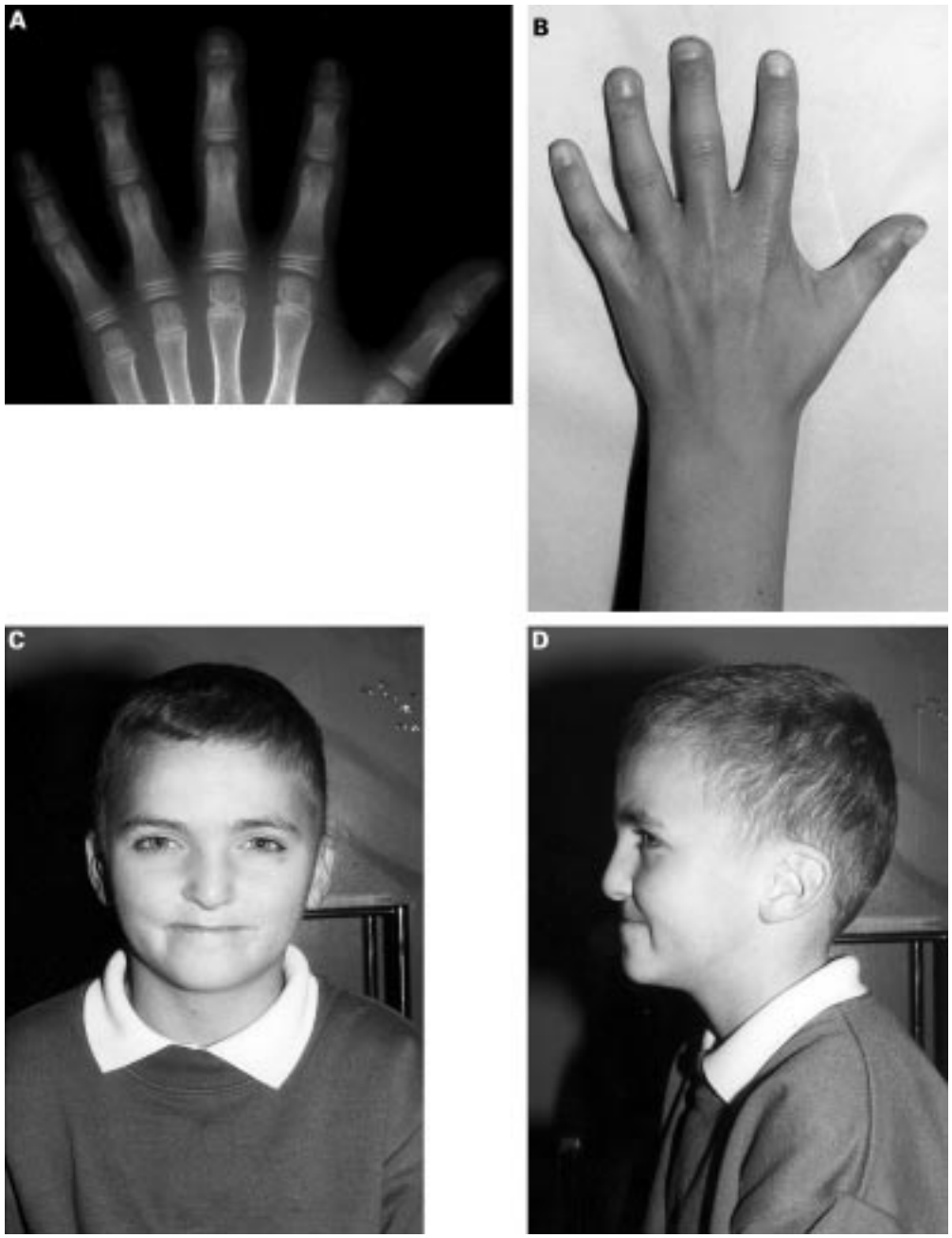

Figure 2 (A) Radiograph of left hand showing shortening of distal phalanx of middle finger. (B) Dorsum of left hand. (C) AP and (D) lateral views of our patient, aged 9, showing facial dysmorphism similar to that in Binder syndrome (see text for description).

the past 10 years on patients with distal Xp deletions imply, however, that the putative X linked mental retardation (XLMR) gene, *MRX49*, lies distal to *GS1* and *STS* but proximal to DXS31 and *CDPX1* (fig 1).[1–4]

Here we describe the clinical, cytogenetic, and molecular features of a boy with an unbalanced X;Y translocation resulting in a deletion of Xp extending from Xptel to the *STS* gene who, intriguingly, does not have learning difficulties (LD), despite the loss of this putative XLMR locus.

Case report

This 9 year old boy was delivered at term by caesarean section on account of fetal distress. He weighed only 2610 g but did not have any significant problems neonatally. His developmental milestones were achieved satisfactorily but he was investigated, aged 21 months, on account of his significant hyperactivity and ichthyosis. He has facial dysmorphism akin to that of Binder syndrome, including a broad nasal bridge and forehead, maxillary hypoplasia, relative prognathism, and dental malocclusion, in addition to terminal phalangeal shortening (fig 2). He suffered from epileptic seizures from the age of around 6 months, requiring prophylactic medication for two years. With the exception of one seizure that lasted 45 minutes, the fits were all brief and associated with pyrexia. His height lies just above the 3rd centile, while his head circumference and weight lie between the 25th and 50th centiles. He was diagnosed by a child

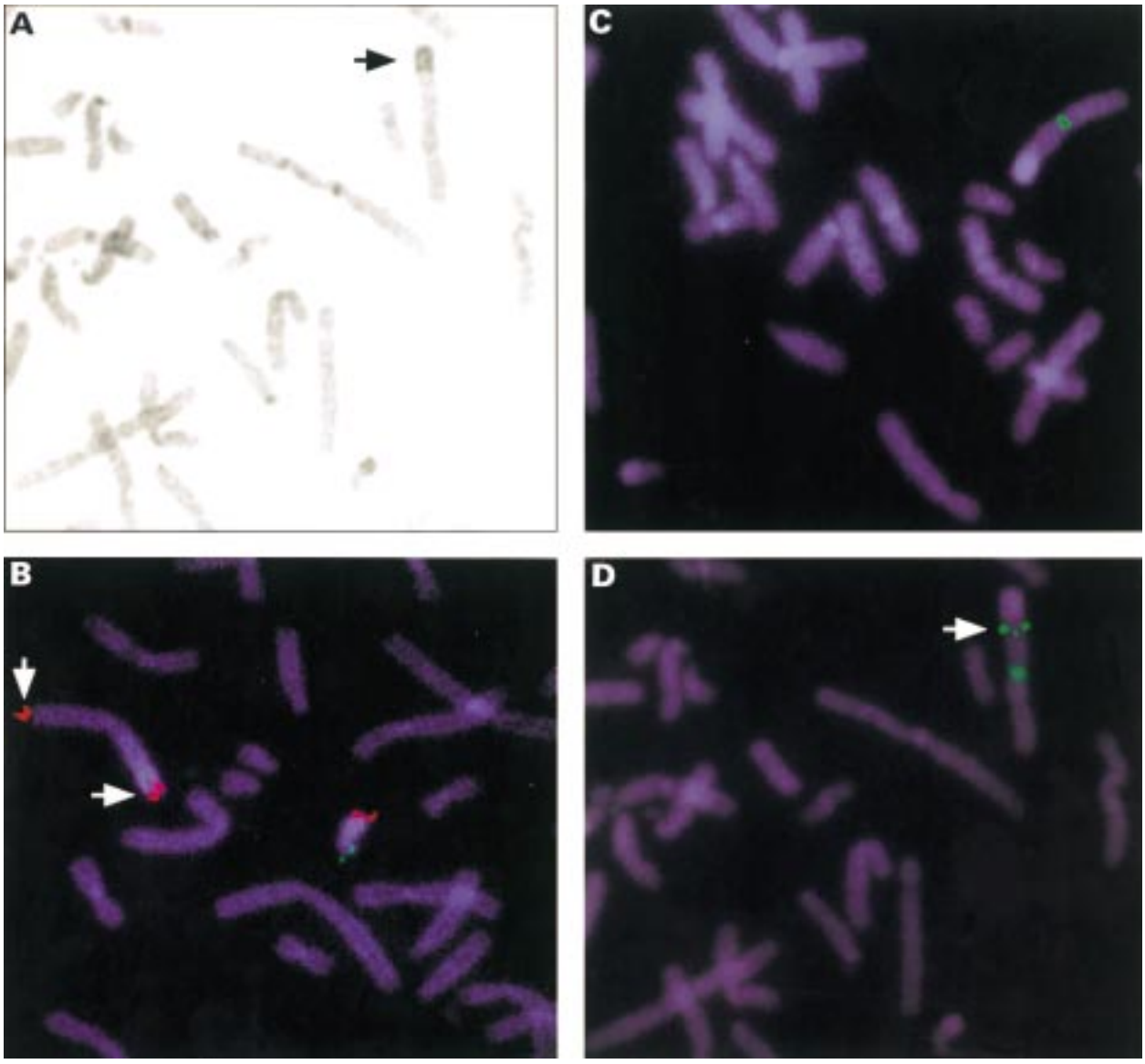

Figure 3 Chromosome analysis of our patient. (A) DAPI stained metaphase spread showing the presence of Y derived heterochromatin (arrow) on Xp. FISH analysis showing (B) the presence of the (red) XYqtel signal (arrow) at the tips of both arms of the derivative X chromosome and the (green) XYptel signal on the Y chromosome only, (C) the absence of STS signal on the derivative X chromosome, and (D) the presence of KAL1 signal (arrow) on the derivative X chromosome.

psychiatrist as having attention deficit hyperactivity disorder (ADHD) and was treated successfully with methylphenidate.

Psychometric testing, done by a senior educational psychologist, in addition to his assessment by his schoolteacher, indicated that he is of average cognitive potential and does not have any innate learning difficulties. His CT

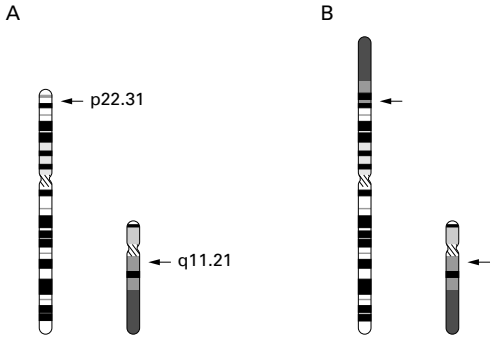

Figure 4 Schematic indication of the origin of the derivative X chromosome showing (A) the X and Y chromosome breakpoints and (B) the derivative X chromosome.

Table 1 Results of molecular (DXYS233, PABX, DXS996, DXS1118E, DXS6837, DXS1139, DXS6834, DXS1130, DXS237, DXS278, and DXS987) and FISH (XYqtel, XYptel, STS and KAL1) analyses undertaken to determine the location of the Xp breakpoint in our patient

Marker	*Result*
XYqtel	+
XYptel	−
DXYS233	−
PABX	−
DXS996	−
DXS1118E	−
DXS6837	−
DXS1139	−
DXS6834	−
DXS1130	−
STS	−
DXS237	−
DXS278	+
KAL1	+
DXS987 (Xp22.2)	+

and EEG proved normal. Cytogenetic analysis, however, showed his karyotype to be 46,Y,der(X)t(X;Y)(p22.31;q11.21). The derivative X chromosome was found to lack the XYptel and *STS* sequences but to contain a large region of Y long arm material, including the heterochromatic region and the XYqtel sequence (figs 3 and 4). Additional FISH and molecular analyses were carried out, localising the Xp breakpoint to between the *STS* and *KAL1* genes (table 1). His mother is a carrier of the derivative X chromosome and has carrier levels of STS activity.

All FISH probes were used essentially according to the manufacturer's instructions. Hybridisations were performed on metaphase chromosomes using *STS* or *KAL1* Xp22.3 region probe with DXZ1 chromosome X control probe (ONCOR)[1 5] or chromoprobe T XYptel/qtel (CYTOCELL).[6]

Discussion

While the boy's short stature, craniofacial abnormalities, and ichthyosis are certainly consistent with the loss of the *SHOX*, *CDPX1*, and *STS* genes, respectively, the severe hyperactivity which he exhibited in his early childhood was not readily predictable from his karyotype. Although both twin and adoption studies suggest that attention deficit and hyperactivity are strongly heritable,[7] these complex disorders are likely to be multifactorial and genetically heterogeneous. The marked hyperactivity observed in this boy and the boy reported by Spranger *et al*[4] might reflect the loss of an unidentified ADHD susceptibility gene in this Xp region, although we cannot exclude the possibility of the ADHD being an unrelated finding.

The results of the mapping studies (fig 1) have hitherto been interpreted as indicating that an XLMR gene is located between *DXS31* and *STS*. These analyses have included a clinical and molecular study of 27 patients with deletions involving the distal short arm of the X chromosome,[1] a description by Schaefer *et al*[3] of several patients with LD and terminal and interstitial Xp deletions,[3] and a two point linkage analysis with X chromosomal markers on a family in which five males in two generations showed mild to moderate LD.[8]

The boy reported by Spranger *et al*,[4] with an Xp terminal deletion with a breakpoint distal to the *STS* gene, was described as having LD in addition to short stature and chondrodysplasia punctata. Their molecular analysis would suggest that the putative MRX gene, *MRX49*, lies distal to *GS1*, which is consistent with the mapping data provided by Ballabio *et al*[1] (fig 1). Furthermore, very recently, a gene which resides between markers DXS1139 and DXS6837, *VCX-A*, was identified by further deletion mapping of 15 males with Xp deletions.[9] This gene was reported to be deleted or retained in all of the subjects who had LD or were of normal intelligence, respectively.[9]

The lack of LD in the boy described here would suggest, however, either that the putative XLMR gene is located more proximally than previously considered or that if such a gene is located distal to *STS*, its deletion is alone insufficient to cause LD. The genotype-phenotype correlation is, therefore, much less straightforward than might have been inferred from previous reports. This has important implications for the accurate counselling of carriers of similar Xp chromosomal deletions.

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Identification of a new *TWIST* mutation (7p21) with variable eyelid manifestations supports locus homogeneity of BPES at 3q22

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EDITOR—Blepharophimosis-ptosis-epicanthus inversus syndrome (BPES) is an autosomal dominant disorder of eyelid development defined by small palpebral fissures, epicanthus inversus, and ptosis.[1 2] BPES type I (OMIM 110100) is characterised by female infertility, whereas BPES type II (OMIM 601649) is transmitted by both females and males. Most cases of BPES types I and II map to chromosome 3q22-q23 (*BPES1*).[3–7] However, a second locus (*BPES2*) was reported in the chromosome 7p13-p21 region on the basis of patients presenting with eyelid anomalies carrying chromosomal abnormalities in the 7p21 region[8–11] and the further linkage data of a large Indian family diagnosed initially with BPES type II.[7] The *TWIST* gene, mapped on chromosome 7p21, codes for a transcription factor with a bHLH domain.[12] *TWIST* mutations[13–18] have been reported in the heterozygous state in patients presenting with the Saethre-Chotzen syndrome (SCS, OMIM 101400). This disorder is a common autosomal dominant form of syndromic craniosynostosis defined by craniostenosis, minor limb and ear abnormalities, and frequent ptosis of the eyelids.[19] In the present study, molecular genetic analysis at *TWIST* and subsequent clinical re-evaluation of the Indian family were used to investigate the possibility that prominent eyelid malformations may represent a clinical variant in the spectrum of phenotypes associated with SCS.

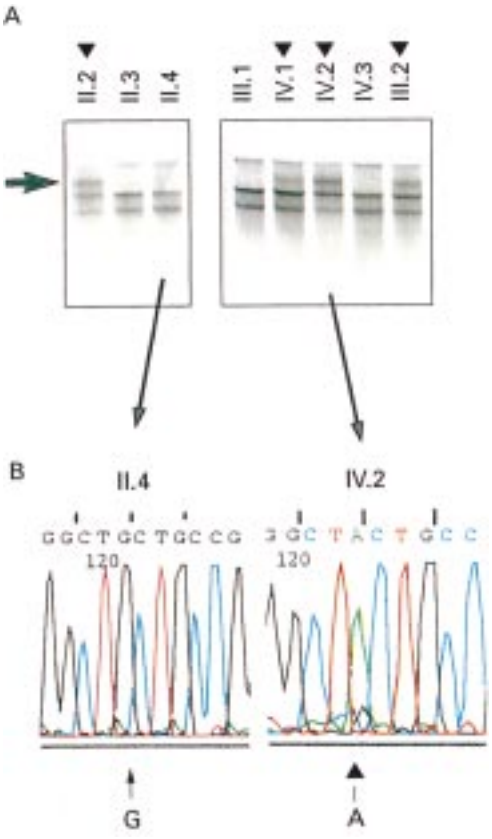

Figure 1 Abnormal SSCP pattern and sequence analysis showing the TWIST mutation (identification of patients was done according to Maw et al[7]). (A) Abnormal SSCP band (arrow) of affected patients (arrow heads) compared to unaffected members of the family. (B) Sequence analysis: antisense sequence of II.4, an unaffected member of the family, compared to IV.2, a patient carrying the mutation. All the patients with an abnormal SSCP band carried the 28 C→T mutation.

The four generation Indian family originates from the Bihar region in the western part of India. The members of the family were initially referred in 1995 because of palpebral anomalies.[7] Clinical re-evaluation of the family took place at the Anandalok Eye Hospital in Calcutta in June 2000. Nineteen members of the family, including 17 affected persons, were examined in detail (photographs and *x* rays available on request for all affected members).

DNA samples, extracted from Guthrie cards, were available from 31 members of the Indian family.[7] These samples were PCR amplified for *TWIST* and PCR products were subjected to single strand sequence conformation polymorphism (SSCP) and direct sequencing analyses. The primer pair that allowed detection of the mutations reported here was forward primer, VB56 (5' - GAG GCGCCCCGCTCTTCTCCTCTG - 3') and reverse primer TQ 53 (5' - CGTCTGAAGAACGGCGCGAA - 3'). A specific migration pattern was observed after amplification of the DNA of all 16 members of the Indian family who were previously reported to have an abnormal clinical examination. In all cases, the abnormal SSCP pattern cosegregated with the previously reported haplotype of linked chromosome 7p markers[7] (fig 1). Direct sequence analyses were performed on the PCR products of all 31 DNA samples. All 16 samples with an abnormal SSCP pattern showed the same heterozygous mutation, namely a C to T transition at position +82, changing a CAG codon to TAG, which is predicted to result in premature termination of the protein at codon 28 (28 C→T) positioned far upstream of the bHLH motif and probably within the recently reported histone acetyltransferase interaction domain.[20] This nonsense *TWIST* mutation probably results in the absence of stable protein synthesis from the