Am. J. Hum. Genet. 56:907–914, 1995

Overlapping Submicroscopic Deletions in Xq28 in Two Unrelated Boys with Developmental Disorders: Identification of a Gene Near FRAXE

Á. K. Gedeon,[1,3] M. Keinänen,[5] L. C. Adès,[2] H. Kääriäinen,[6] J. Gécz,[1] E. Baker,[1] G. R. Sutherland,[1,4] and J. C. Mulley[1]

Departments of [1]Cytogenetics and Molecular Genetics and [2]Medical Genetics, Centre for Medical Genetics, Women's and Children's Hospital, North Adelaide; Departments of [3]Genetics and [4]Pediatrics, University of Adelaide, Adelaide; [5]Clinical Laboratory Medix, Espoo, Finland; and [6]Department of Medical Genetics, University of Helsinki, Helsinki

Summary

Two unrelated boys are described with delay in development and submicroscopic deletions in Xq28, near FRAXE. Molecular diagnosis to exclude the fragile X (FRAXA) syndrome used the direct probe pfxa3, together with a control probe pS8 (DXS296), against *Pst*I restriction digests of DNA. Deletions were detected initially by the control probe pS8, which is an anonymous fragment subcloned from YAC 539, within 1 Mb distal to FRAXA. Further molecular analyses determined that the maximum size of the deletion is <100 kb in one boy (MK) and is wholly overlapped by the deletion of up to ~200 kb in the other (CB). These deletions lie between the sequences detected by the probe VK21C (DXS296) and a dinucleotide repeat VK18AC (DXS295). The patient MK had only speech delay with otherwise normal development, while patient CB had global developmental delay that included speech delay. Detection of overlapping deletions in these two cases led to speculation that coding sequences of a gene(s) important in language development may be affected. Hybridization of the pS8 and VK21A probes to zooblots revealed cross-species homology. This conservation during evolution suggested that this region contains sequences with functional significance in normal development. The VK21A probe detected a 9.5-kb transcript in placenta and brain and a smaller, 2.5-kb, transcript in other tissues analyzed.

Introduction

Three folate-sensitive fragile sites have been cloned in Xq27-q28: FRAXA in Xq27.3 and FRAXE and FRAXF in Xq28 (Oberlé et al. 1991; Yu et al. 1991; Knight et al. 1993; Parrish et al. 1994). The fragile X syndrome, associated with the FRAXA fragile site, is the most common form of X-linked mental retardation. The mutation found in the majority of patients with fragile X syndrome is unstable amplifications of the trinucleotide $(CCG)_n$ repeat within the 5′ UTR of the FMR1 coding sequence (Verkerk et al. 1991). Abnormal expansion of the repeat beyond ~230 copies extinguishes expression of the FMR1 gene (Pieretti et al. 1991). Rare patients with the clinical features of the fragile X syndrome but with no CCG amplification and no cytogenetic expression of a fragile site have loss of gene function, due either to submicroscopic deletions removing all or part of the FMR1 gene (Gedeon et al. 1992; Wöhrle et al. 1992; Tarleton et al. 1993; Meijer et al. 1994; Trottier et al. 1994) or to point mutation (de Boulle et al. 1993). Correlation of the nonspecific mental retardation phenotype to repeat expansion at FRAXE is less conclusive (Knight et al. 1993; Mulley et al., in press), and FRAXF has no apparent association with mental impairment (Parrish et al. 1994).

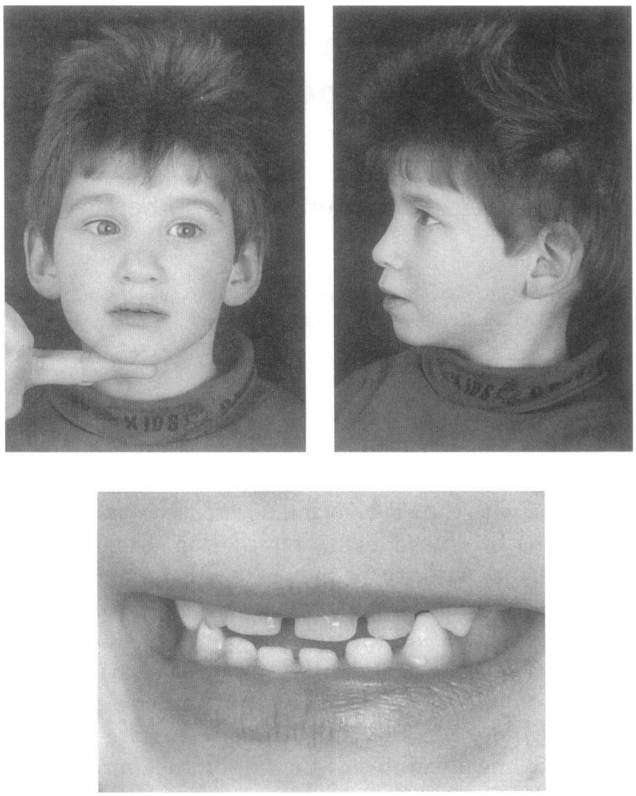
The clinical features of fragile X syndrome, although recognizable in older patients, are subtle in very young individuals, in whom the syndrome can elude early detection. One of the first nonspecific signs is developmental delay, and, for this reason, children with developmental delay are referred for diagnosis of the fragile X syndrome. Molecular diagnosis relies on Southern analysis using probes such as pfxa3, StB12.3, and Ox1.9, which detect the amplification of the FRAXA CCG repeat. The pfxa3 diagnostic protocol (Sutherland et al. 1991) includes the anonymous probe pS8, at the DXS296 locus, to act as an internal control that confirms the presence of fully digested DNA in each sample track.

Australian patients with developmental delay were screened with these probes for early ascertainment of cases with fragile X syndrome. Deletion of the pS8 control probe was detected in one case, and a second case was independently ascertained in Finland by using the same probe combination. Both deletions are distal to

Received November 4, 1994; accepted for publication January 19, 1995.

Address for correspondence and reprints: Dr. Ági K. Gedeon, Department of Cytogenetics and Molecular Genetics, Women's and Children's Hospital, North Adelaide, 5006, South Australia.

0002-9297/95/5604-0012$02.00



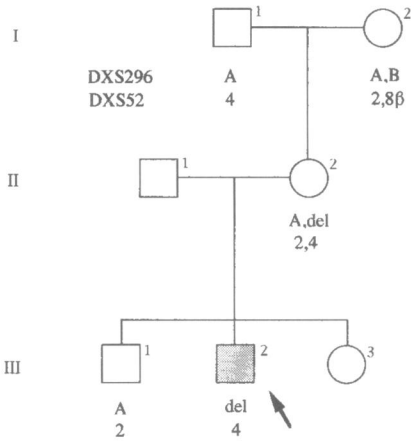


Figure 1 *Left,* Face of patient CB. Note the widely spaced teeth and bilateral epicanthal folds. Clinical assessment of this boy at age 2 years 9 mo revealed global developmental delay. *Above,* Pedigree of CB (III-2). The proband is indicated by an arrow. DXS296 (VK21A-*Taq*I RFLP) and DXS52 genotypes are shown (DNA was not collected from II-1 or III-3). II-2 was determined to carry the deletion by dosage and was confirmed to be hemizygous rather than homozygous for allele A at DXS296, by FISH.

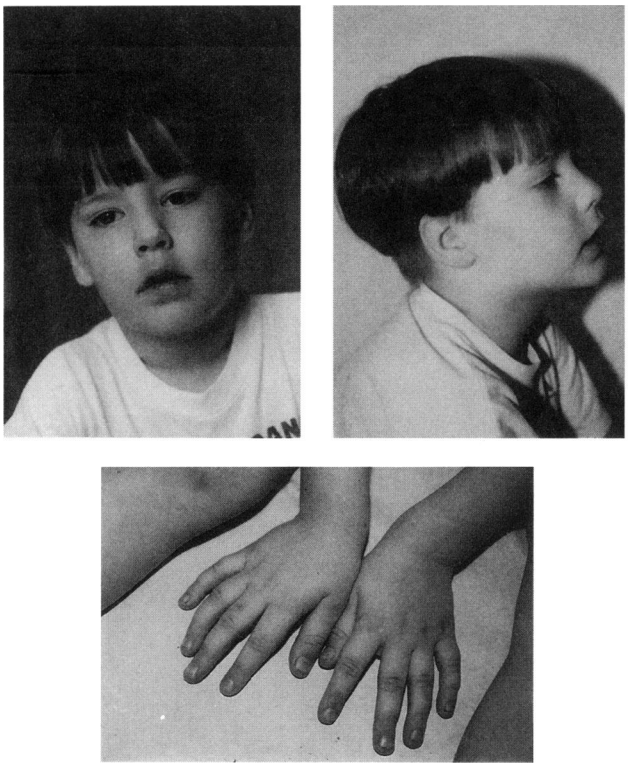
FRAXA and FRAXE and do not involve the FMR1 gene. We present the clinical and molecular findings in these two cases and their available families, and we also present evidence that suggests the presence of a gene within these deletions that is involved in the development of normal brain function.

Patients, Material, and Methods

Patients

Patient CB (Australia).—The proband (fig. 1, *left-hand panels*) was born at 34 wk gestation, with hyaline membrane disease. He is the second child of nonconsanguineous parents (fig. 1, *right-hand panel*). The elder brother is normal in intellect and development. Physical examination of the proband, at age 2 years 9 mo (corrected age 2 years 7 mo), showed mild joint hyperextensibility and generalized hypotonia. Speech and motor skills were delayed. He was an affectionate child who displayed excessive hand flapping and tremulousness when excited. He had grown normally, with height 96.5 cm (75th centile), weight 13.7 kg (25–50th centile), and head circumference 49.5 cm (50th centile), but he had slight dysmorphism, with protuberant ears, bilateral epicanthal folds, a divergent strabismus, and widely spaced upper and lower teeth (fig. 1, *left-hand panels*). There was a small telangiectasia over the lateral aspect of the left nostril, as well as a flat hemangioma at the nape of the neck. The palate, hair, and hairline were normal, as were the fingers, nails, and palmar creases. Neurological, cardiovascular, and genital findings were normal. Formal assessment revealed a global developmental delay of 18 mo.

Patient MK (Finland).—This boy (fig. 2, *left-hand panels*) who had speech delay, is the second child (fig. 2, *right-hand panel*) of three boys. Both of his brothers have developed normally; the younger brother appears normal at the age of 2 years. The parents were healthy and nonconsanguineous. The mother's maternal aunt has a 13-year-old son who has poor speech but attends normal school, with major difficulties. Clinical assessment of the patient MK at 4 years 9 mo showed that his motor skills and neurological performance were normal. His motor development was normal, he walked unaided at 10 mo of age, and he was not clumsy or hypotonic. He had grown normally in height (97th centile), and his weight was normal for height and head circumference (75–97th centile). He had mild dysmorphic features (fig. 2, *left-hand panels*), with slight epicanthal folds, long eyelashes, and a somewhat higher nasal bridge than seen in the average Finnish child (Finnish children usually have a low nasal bridge). The skin, hair, hairline, palate, teeth, and ears were normal. The fifth fingers (fig. 2, *left-hand panels*) and the second toes showed mild clinodactyly. Cardiovascular, respiratory, abdominal, and genital examination findings and electroencephalogram were normal. MK has delayed speech and, according to his parents, severe difficulties in understanding speech.



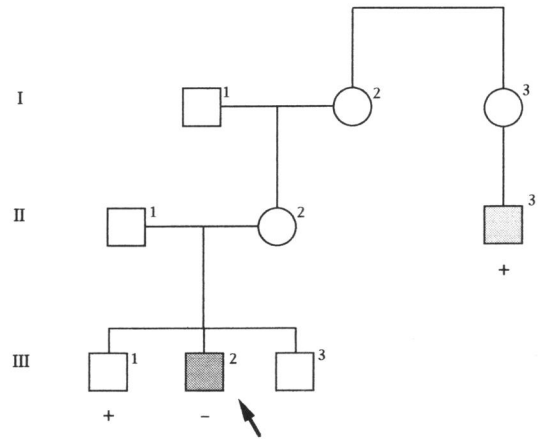


Figure 2 *Left,* Face and hands of patient MK, deleted for pS8 sequence. Clinical assessment showed isolated speech delay only. *Above,* Pedigree of MK. The proband is indicated by the arrow. The presence (+) or absence (−) of the pS8 sequence is indicated for family members, where known.

Audiological evaluation, undertaken because of the speech delay and poor comprehension, was normal at age 4 years. During the genetic counseling session he said some words that only the parents could understand. He is said to have a habit of rolling himself on the floor in a nearly autistic fashion, although he stops when told to. He performs within the average normal range on psychological tests (Leiter, WPPSI, and VMI), except on tests needing verbal skills.

Material and Methods

Molecular analysis.—Molecular diagnosis for the fragile X syndrome was carried out using the pfxa3 and pS8 probe combination (fig. 3), as described elsewhere (Sutherland et al. 1991). The probes pS8, VK21A, and VK21C represent the DXS296 locus and map onto YAC539 (fig. 4*A*). The VK21 subclones of DXS296 map to Xq28, ~600 kb distal to FRAXA (Bell et al. 1991) and also distal to FRAXE, but ~800 kb proximal to the IDS gene (Wilson et al. 1991). The sequence at DXS295 (VK18AC), amplified by PCR, maps only to the distal YAC D49G8, while pS8 and VK21A link three YACs—XY539, A14D5, and D49G8—into a contig spanning >500 kb (Kalatsis 1991). Oligonucleotide primer pairs XY539R and XY539L at the right and left ends, respectively, of the YAC539 were available for amplification of PCR products, to define the deletions. The more telomeric probe at DXS52 (St-14) was also analyzed, in order to determine chromosomal phase of the deletion in CB. The probe OxE20 was used against *Hin*dIII digests, to determine if this sequence near the FRAXE CCG repeat was involved in the deletions. PCR amplifications and Southern analyses were performed as described elsewhere (Gedeon et al. 1992).

*Eco*RI-digested DNA from the somatic cell hybrids,

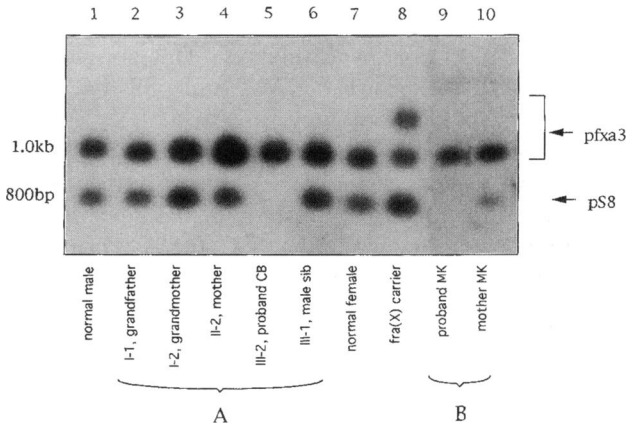


Figure 3 Southern analysis using pfxa3 and pS8 double hybridization on *Pst*I-digested DNA from (*A*) the family of CB and (*B*) the patient MK and his mother. The normal band detected by pfxa3 is 1.0 kb, and that detected by pS8 is 800 bp. The control probe pS8 is deleted from CB (lane 5) and MK (lane 9). In *A*, normal males are in lanes 1 (unrelated control), 2 (I-1), and 6 (III-1). The grandmother (I-2), lane 3, has the same relative dosage of pfxa3 to pS8 as does the unrelated female control in lane 7, but the mother of CB (lane 4) has only half the relative intensity of pS8 to pfxa3, indicating that she is a carrier. In *B*, the carrier status of the mother of MK (lane 10) can be determined in the same way.

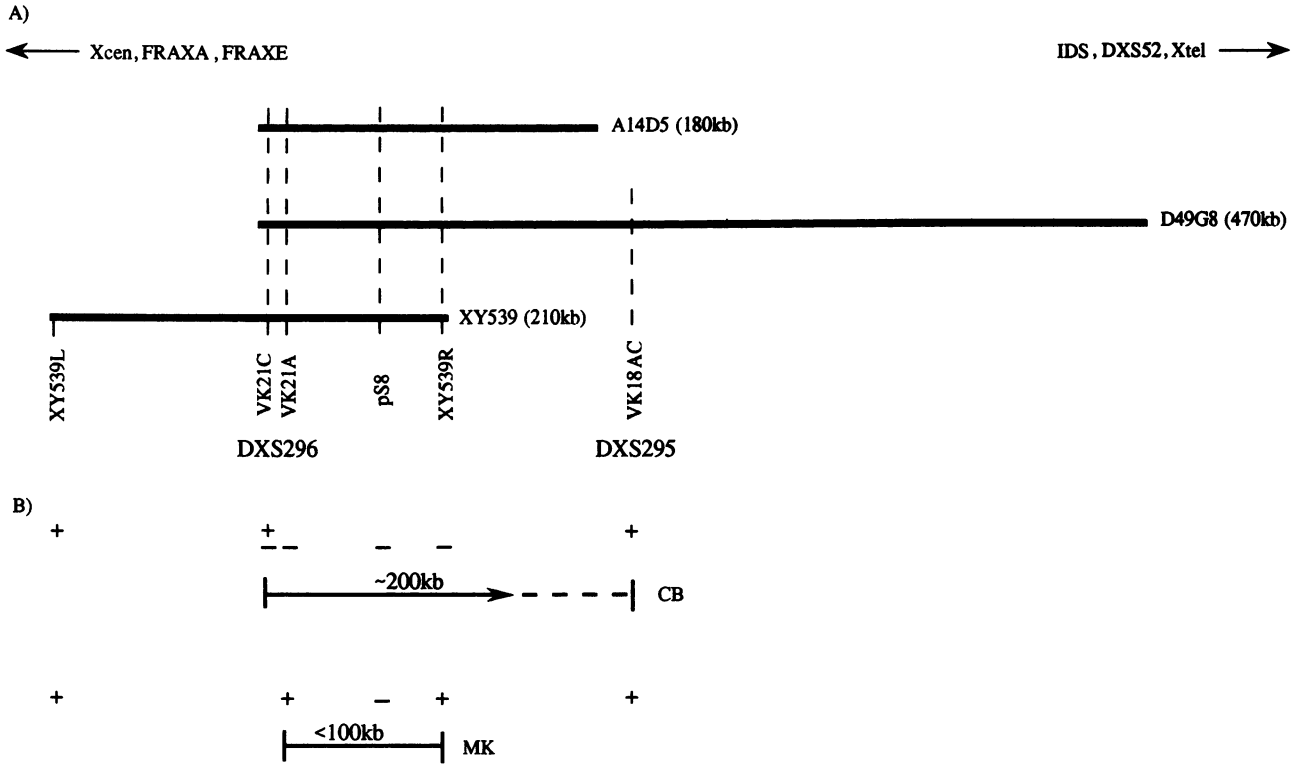


Figure 4 Linear map of the region. *A*, Relative positions of probes VK21A, VK21C, and pS8 and PCR primers XY539L, XY539R, and VK18AC are shown against overlapping YACs XY539, D49G8, and A14D5 and with respect to the orientation on the X chromosome. *B*, Extent of the deletion in each patient, with presence (+) or absence (−) of marker sequences noted for each probe.

CY2 and CY3, containing complementary portions of the X chromosome that were derived from a human X:16 translocation in a mouse background (Suthers et al. 1990), were included on a zooblot, together with samples from mouse, dog, chicken, man, echidna, orangutan, marmoset, and monkey. This zooblot was screened with the probes VK21A and pS8 (fig. 5), to detect cross-species homology.

Northern blot hybridization(s), using both pS8 and VK21A probes, was carried out according to the instructions of the manufacturer of the blot (Clontech). After a medium-stringency wash (2 × SSC, 0.5% SDS at 42°C for 15 min, for both probes), the membrane was exposed to an autoradiography film (Kodak) at −80°C for 48–96 h.

Cytogenetic and FISH analyses.—Routine cytogenetic analysis for chromosomal abnormalities and fragile sites was carried out on a peripheral blood sample from patient CB. FISH was used to determine the carrier status of his sister (III-3). Hybridization with the combined probes VK21A and pS8 was performed as described elsewhere (Callen et al. 1990). A plasmid hybridizing at high stringency to the pericentromeric alphoid sequences of the X chromosome (Choo et al. 1987) was also added to the hybridization mixture.

To assess the pattern of X inactivation in CB's carrier mother (II-2), FISH was performed on R-banded metaphases exhibiting early- and late-replicating X chromosomes, by using a method described elsewhere (Gedeon et al. 1992) and the same three probes mentioned above.

Results

The pfxa3 probe was unremarkable in either boy, with each demonstrating the normal 1.0-kb *Pst*I fragment (fig. 3). This excluded the diagnosis of fragile X syndrome but confirmed the presence of fully digested DNA in each lane. The lack of signal observed with the pS8 probe therefore indicated submicroscopic deletions in each boy. Further investigations, with nearby probes and primer sequences, characterized the extent of the deletion in each patient (fig. 4*B*). Family studies were undertaken to determine whether these deletions were de novo or inherited mutations. Neither boy had either abnormal CCG expansion at the FRAXE locus or alteration of the 5.2-kb *Hin*dIII fragment present in normal boys, as determined by the OxE20 probe.

Patient CB

This patient did not have detectable cytogenetic expression of a fragile site or a microscopically visible chromosomal deletion. The pS8 probe detects an 800-bp *Pst*I fragment or a 6.0-kb *Hin*dIII fragment in normal individuals. Neither fragment was present in this boy.

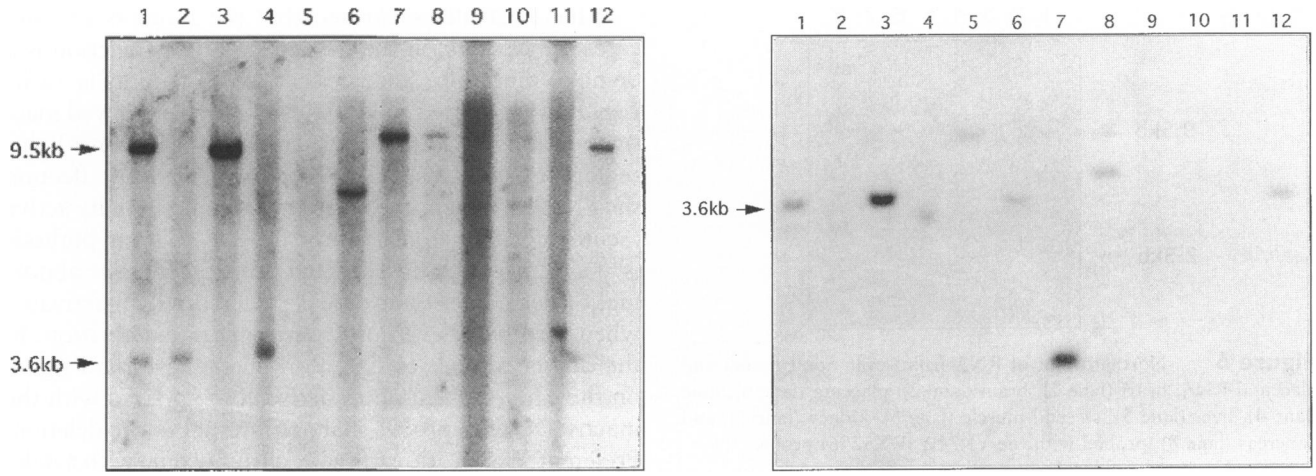


Figure 5 Zooblot with *Eco*RI-digested DNA from somatic cell hybrids in a mouse background. Shown are CY3 (lane 1), CY2 (lane 2), and samples representing several species: human (lane 3), mouse (lane 4), hamster (lane 5), dog (lane 6), marmoset (lane 7), monkey (lane 8), orangutan (lane 9), chicken (lane 10), echidna (lane 11), and human (lane 12). Evolutionary conservation of the pS8 sequence (*left*) and the VK21A sequence (*right*) is indicated by hybridization of these probes across several species.

Sequences complementary to the probe VK21A and the distal end clone from YAC539 (XY539R) were also deleted in the proband. The probe VK21C detected only one of the two expected monomorphic *Taq*I fragments of 5.9 and 5.2 kb. Absence of the 5.2-kb fragment defines the proximal breakpoint for this deletion. The distal breakpoint could not be as accurately determined. The dinucleotide sequence of VK18AC at DXS295, distal to DXS296, was not deleted and is therefore outside the telomeric limit to the deletion. The distal breakpoint for this deletion lies in the interval between XY539R, at the distal (right) end of YAC539, and DXS295. This submicroscopic deletion spans 100–200 kb and is associated with, though is not necessarily the cause of, global developmental delay in this boy.

Family studies determined that the VK21A probe sequence was present in the grandfather (I-1) and the normal male sibling (III-1). The *Taq*I RFLP was heterozygous in the grandmother (I-2) (fig. 1, *right-hand panel*). Genotyping of the DXS52 RFLP within this family was consistent with the pedigree as given and suggested that the deletion arose in the grandpaternal chromosome and was transmitted as a new mutation to II-2 (fig. 1, *right-hand panel*). Dosage estimation (fig. 3*A*), by comparison of the relative intensities of pfxa3 fragments to pS8 fragments within each lane, demonstrated that the mother (II-2) carries the deletion. The relative ratio of the pS8 fragment to the pfxa3 fragment is approximately halved in II-2.

The result of FISH to chromosomes from the proband's sister III-3 showed hybridization of the probes VK21A and pS8 (internal to the deletion) to both X chromosomes, demonstrating that she did not carry the deleted chromosome. FISH applied to the maternal chromosomes from blood lymphocytes, with probes internal to the deletion (VK21A and pS8), gave signal on only one Xq in 60 metaphases examined. The signal was on the active X in 15 of these 60 metaphases.

Patient MK

Although deleted for the *Pst*I fragment detected by the pS8 probe, the flanking sequences VK21A and more proximal XY539L, as well as XY539R and distal DXS295, were retained on the X chromosome of this boy. The deleted region is >800 bp and <100 kb. It is completely overlapped by the deletion in patient CB. The elder brother of MK did not carry the deletion, and the youngest sibling was not tested. Dosage analysis based on the relative intensity of the pfxa3 fragments to the pS8 fragments in his mother (fig. 3*B*) suggested that she was a carrier, but this could not be confirmed without FISH analysis or an informative polymorphic marker within the interval. A junction fragment at the telomeric breakpoint of this deletion confirmed that the mother of MK carries this deletion (J. Gécz, unpublished data). The mother's cousin (II-3), although slow in developing speech, does not carry the deletion.

Conserved Sequences

The pS8 sequence was shown to be conserved across several species (fig. 5, *left*). This unique-copy probe detects a 9.5-kb *Eco*RI fragment in human and a 3.6-kb fragment in mouse. In the hybrid cell line CY2, containing the human X chromosome from Xpter-Xq26 in a mouse background, only the mouse band (3.6 kb) can be seen and is the same size as the band seen in lane 4, which is mouse cell line A9. The reciprocal product of the X:16 translocation in CY3 contains the Xq26-qter portion of the X chromosome. In CY3, the human band is visible, as is the mouse-specific band. Reprobing of

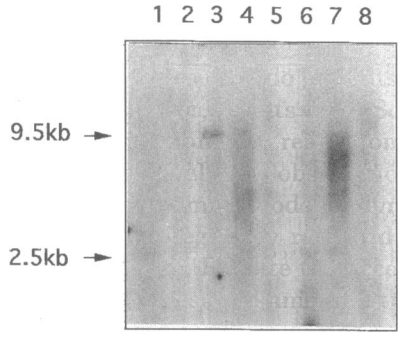


Figure 6 Northern blot of RNA from seven adult tissues and placental RNA: heart (lane 1), brain (lane 2), placenta (lane 3), lung (lane 4), liver (lane 5), skeletal muscle (lane 6), kidney (lane 7), and pancreas (lane 8), probed with the VK21A (DXS296) probe.

the zooblot with VK21A (fig. 5, *right*) revealed a similar pattern of homologous bands.

Northern Blot Analysis

Of the two probes VK21A and pS8, only VK21A identified a transcript on a northern blot of seven adult-tissue and placental RNAs. The VK21A-associated gene was found to be expressed as an ~9.5-kb transcript, predominantly in placenta and at a lower level in brain (fig. 6). In addition to the 9.5-kb transcript, a smaller-size transcript, ~2.5 kb, was found to be present in the other tissues, including heart, liver, skeletal muscle, and kidney (fig. 6). Whether these represent an alternatively spliced 9.5-kb transcript or another transcript(s) from an additional gene(s) associated with the VK21A probe has not been determined.

Discussion

Submicroscopic deletions of the pS8 sequence were detected in two unrelated boys with mild clinical phenotypes. Patient CB was referred for FRAXA analysis, as a consequence of his "developmental delay." His clinical status may not be related to the pS8 deletion; however, presentation of another case, MK, also deleted for pS8 and with delay in speech development, adds credence to the hypothesis that, within these deletions, there may be coding sequences that are responsible for a previously undescribed nonspecific phenotype with mild mental impairment or developmental problems.

The clinical phenotype in both boys is distinct from that seen in patients with fragile X syndrome and is less severe. Both boys have nonspecific features, with bilateral epicanthal folds, speech delay, and minor behavioral traits as the common features. There is no cytogenetic or molecular evidence for involvement of the FMR1 gene, which maps proximal to DXS296. The deleted region is >600 kb distal to FRAXA (Bell et al. 1991), and the 17 exons of FMR1 span only 38 kb of genomic DNA (Eichler et al. 1993).

It has been demonstrated that the mothers of both cases are carriers. In the mother of CB the deletion is a de novo mutation, since neither of her parents is a carrier. FISH was used to investigate whether skewed inactivation was a feature associated with deletion of this region. It has been suggested that, for deletions affecting the FRAXA region, the deleted X is preferentially active (Schmidt et al. 1990; Clarke et al. 1991). This hypothesis is at variance with the accepted principle that the abnormal or deleted chromosome is preferentially inactivated when there is a deviation from random inactivation. In the carrier mother of CB, more metaphases had signal on the early-replicating or active X, compared with the inactive X, with probes that are internal to the deletion. Preferential activation of the X chromosome with a deletion of this region was therefore not supported and was in agreement with results of a previous study involving a deletion of FMR1 (Gedeon et al. 1992).

The FRAXE CpG island is estimated to be ~150 kb proximal to DXS296 (Knight et al. 1993). The PFGE map of YAC539 (Yu 1992), does not indicate evidence of a CpG island near the pS8 clone, but a *Bss*HII site ~25 kb distal to XY539L, which is ≥75 kb proximal to the DXS296 (VK21) locus, is likely to be the same *Bss*HII site shown by Knight et al. (1993) as being ~100 kb distal to the FRAXE CpG island. It cannot be ascertained, without further physical mapping, whether the FRAXE gene extends toward and onto the YAC XY539. Although the probe OxE20 is not involved in the largest deletion, the close proximity of the FRAXE gene region, adjacent to DXS296, should not be overlooked. This result does not exclude involvement of a gene associated with the FRAXE CpG island, since such a gene may extend over many kilobases of genomic DNA. The cDNA associated with the FRAXE CpG island has not been isolated and thus is not yet available for examination of alterations in the transcript in these patients.

The fact that there was mental retardation in patient CB but that it was not seen in patient MK may be a consequence of the respective sizes of the deletions. The deletion in the case of CB may involve additional genes or possibly part of the coding sequence of the same gene that is disrupted by CCG instability in FRAXE-positive individuals. Alternatively, the mental retardation may arise because of faulty transcription of another gene(s) in close proximity to FRAXE. Association with global developmental and speech delay in CB and with isolated speech delay in MK suggests that a gene with a functional role in normal language development may be found within the common deleted sequences near pS8. Deletions of the IDS gene have been shown to extend proximally to include the DXS296 locus, although the severity of the phenotype of Hunter syndrome would make it difficult to identify other minor physical or intellectual abnormalities (Wilson et al. 1991) attributable to other genes associated with deletion of pS8 and VK21A

sequences. A cytogenetically visible deletion of FMR1 extends over 3 Mb distally and includes the DXS296 locus (Tarleton et al. 1993). The phenotype in that case had much in common with CB, in that he had bilateral epicanthal folds, hypotonia, and delayed psychomotor and speech development (Albright et al. 1994). Many of these features are likely to be due to deletion of both FRAXA and FRAXE, so, again, separation of specific features associated only with deletions of VK21A (DXS296) is not possible. Deletions generally represent only a small proportion (5%–10%) of the mutations of most disorders (Ketterling et al. 1994), suggesting that the associated gene, possibly the one carrying the FRAXE CCG repeat, may account for a significant portion of cases affected with developmental delay or mild mental impairment, once the fragile X syndrome has been excluded.

The pS8 subclone, used as the control probe in fragile X diagnosis, detects a sequence conserved across several species. No transcript was detected on the northern blot, however, so that pS8 may lie in an intron with functional significance, or putative coding sequences may be expressed only at very low levels or not at all in the adult and placental tissues examined. Deletion of this region in both boys may be related only to the common phenotype of speech delay with minor dysmorphism.

The demonstration of an ~9.5-kb transcript detectable by VK21A confirms the existence of a large gene that may extend into the 100–200-kb deleted region of CB and that is expressed during fetal development and in the adult brain. The deletion of a portion of this transcript in patient CB may account for his global developmental delay. Exons from the VK21A transcript map on either side of the 800-bp–100-kb deletion in MK (J. Gécz, unpublished data). Intervening exons of this transcript or coding sequences for another gene(s), or gene expression may be modified by position effect.

Sequence homology was previously shown with the VK21 probe, which detects a single *Hin*dIII fragment in both mouse and hamster (Wilson et al. 1991). This was confirmed on *Eco*RI digests, and homology with several other, more distant species, including dog, monkey, and chicken, was demonstrated. Similar cross-species homology patterns observed with both pS8 and VK21A probes suggests that they might detect different exon sequences of the same gene or of different genes. This degree of evolutionary conservation, deletion of these probes in patients with developmental problems, and detection of a transcript from the region arouse speculation that these probes represent parts of a gene(s) involved in normal development, which, when inactivated, has clinical significance.

Acknowledgments

This work was supported by the National Health and Medical Research Council of Australia and by an International Research Scholars award from the Howard Hughes Medical Institute to G.R.S. We thank Prof. Kay Davies and Dr. Susan Knight for the OxE20 probe.

References

Albright SG, Lachiewicz AM, Tarleton JC, Rao KW, Schwartz CE, Richie R, Tennison MB, et al (1994) Fragile X phenotype in a patient with a large de novo deletion in Xq27-q28. Am J Med Genet 51:294–297

Bell MV, Hirst MC, Nakahori Y, MacKinnon RN, Roche A, Flint TJ, Jacobs PA, et al (1991) Physical mapping across the fragile X: hypermethylation and clinical expression of the fragile X syndrome. Cell 64:861–866

Callen DF, Baker E, Eyre HJ, Chernos JE, Bell JA, Sutherland GR (1990) Reassessment of two apparent deletions of chromosome 16p to an ins(11;16) and a t(1;16) by chromosome painting. Ann Genet 33:419–421

Choo HK, Brown R, Webb G, Craig IW, Filby RG (1987) Genomic organization of human centromeric alpha satellite DNA: characterization of a chromosome 17 alpha satellite sequence. DNA 6:297–305

Clarke JTR, Greer WL, Strasberg PM, Pearce RD, Skomorowski MA, Ray PN (1991) Hunter disease (mucopolysaccharidosis type II) associated with unbalanced inactivation of the X chromosome in a karyotypically normal girl. Am J Hum Genet 49:289–297

DeBoulle K, Verkerk AJMH, Reyniers E, Vits L, Henrickx J, Van Roy B, Van Den Bos F, et al (1993) A point mutation in the FMR-1 gene associated with fragile X mental retardation. Nat Genet 3:31–35

Eichler EE, Richards S, Gibbs RA, Nelson DL (1993) Fine structure of the human FMR1 gene. Hum Mol Genet 2:1147–1153

Gedeon AK, Baker E, Robinson H, Partington MW, Gross B, Manca A, Korn B, et al (1992) Fragile X syndrome without CCG amplification has an FMR1 deletion. Nat Genet 1:341–344

Kalatzis V (1991) Mapping of contiguous segments of DNA using yeast artificial chromosomes in the region distal to the fragile X. Honours thesis, The University of Adelaide, Adelaide, South Australia

Ketterling RP, Vielhaber EL, Lind TJ, Thorland EC, Sommer SS (1994) The rates and patterns of deletions in the human factor IX gene. Am J Hum Genet 54:201–213

Knight SJL, Flannery AV, Hirst MC, Campbell L, Christodoulou Z, Phelps SR, Pointon J, et al (1993) Trinucleotide repeat amplification and hypermethylation of a CpG island in FRAXE mental retardation. Cell 74:127–132

Meijer H, de Graaff E, Merckx DML, Jongbloed RJE, de Die-Smulders CEM, Engelen JJM, Fryns J-P, et al (1994) A deletion of 1.6 kb proximal to the CGG repeat of the FMR1 gene causes the clinical phenotype of the fragile X syndrome. Hum Mol Genet 3:615–620

Mulley JC, Yu S, Loesch DZ, Hay DA, Donnelly A, Gedeon AK, Carbonell P, et al. FRAXE and mental retardation. J Med Genet (in press)

Oberlé I, Rousseau F, Heitz D, Kretz C, Devys D, Hanauer A, Boue J, et al (1991) Instability of a 550-base pair DNA segment and abnormal methylation in fragile X syndrome. Science 252:1097–1102

Parrish JE, Oostra BA, Verkerk AJMH, Richards CS, Reynolds J, Spikes AS, Shaffer LG, et al (1994) Isolation of a GCC repeat showing expansion in FRAXF, a fragile site distal to FRAXA and FRAXE. Nat Genet 8:229–235

Pieretti M, Zhang F, Fu Y-H, Warren ST, Oostra BA, Caskey CT, Nelson DL (1991) Absence of expression of the FMR-1 gene in fragile X syndrome. Cell 66:817–822

Schmidt M, Certoma A, Du Sart D, Kalitsis P, Leversha M, Foster K, Sheffield L, et al (1990) Unusual X chromosome inactivation in a mentally retarded girl with an interstitial deletion Xq27: implications for the fragile X syndrome. Hum Genet 84:347–352.24

Suthers GK, Hyland VJ, Callen DF, Oberle I, Rocchi M, Thomas NS, Morris CP, et al (1990) Physical mapping of new DNA probes near the fragile X mutation (*FRAXA*) by using a panel of cell lines. Am J Hum Genet 47:187–195

Sutherland GR, Gedeon AK, Kornman L, Donnelly A, Byard RW, Mulley JC, Kremer E, et al (1991) Prenatal diagnosis of fragile X syndrome by direct detection of the unstable DNA sequence. N Engl J Med 325:1720–1722

Tarleton JC, Richie R, Schwartz C, Rao K, Aylsworth AS, Lachiewicz A (1993) An extensive de novo deletion removing FMR1 in a patient with mental retardation and the fragile X syndrome phenotype. Hum Mol Genet 2:1973–1974

Trottier Y, Imbert G, Poustka A, Fryns J-P, Mandel J-L (1994) Male with typical fragile X phenotype is deleted for part of the FMR1 gene and for about 100 kb of upstream region. Am J Med Genet 51:454–457

Verkerk AJMH, Pieretti M, Sutcliffe JS, Fu Y-H, Kuhl DPA, Pizzuti A, Reiner O, et al (1991) Indentification of a gene (FMR-1) containing a CGG repeat coincident with a breakpoint cluster region exhibiting length variation in fragile X syndrome. Cell 65:905–914

Wilson PJ, Suthers GK, Callen DC, Baker E, Nelson PV, Cooper A, Wraith JE, et al (1991) Frequent deletions at Xq28 indicate genetic heterogeneity in Hunter syndrome. Hum Genet 86:505–508

Wöhrle D, Kotzot D, Hirst MC, Manca A, Korn B, Schmidt A, Barbi G, et al (1992) A microdeletion of less than 250 kb, including the proximal part of the FMR-1 gene and the fragile-X site, in a male with the clinical phenotype of fragile X syndrome. Am J Hum Genet 51:299–306

Yu S (1992) Molecular basis of fragile X syndrome. PhD the sis, The University of Adelaide, Adelaide, South Australia

Yu S, Pritchard M, Kremer E, Lynch M, Nancarrow J, Baker E, Holman K, et al (1991) Fragile X genotype characterised by an unstable region of DNA. Science 252:1179–1181