Most Jacobsen Syndrome Deletion Breakpoints Occur Distal to FRA11B

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Recent studies have identified a (CCG)n repeat in the 5' untranslated region of the CBL2 protooncogene (11q23.3) and have demonstrated that expansion of this repeat causes expression of the folate-sensitive fragile site FRA11B. It has also been demonstrated that FRA11B is the site of breakage in some cases of Jacobsen syndrome (JS) involving terminal deletions of chromosome 11q. We report on 2 patients with JS and a 46,XX,del(11)(q23.3) karyotype. In both cases, microsatellite and fluorescence in situ hybridization analyses indicated that the deletion breakpoint was approximately 1.5–3 Mb telomeric to FRA11B. There was no evidence of expansion of the CBL2 (CCG)n repeat in the parents of either patient. The deleted chromosome was of paternal origin in both cases, although it was of maternal origin in the cases reported to be caused by FRA11B. These findings and those in previously reported patients suggest that the breakpoint for most 11q deletions in JS patients is telomeric to FRA11B, which raises the possibility that there may be other fragile sites in 11q23.3 in addition to FRA11B. These findings also support previous evidence that there may be a propensity for breakpoints to differ depending on the parental origin of the deleted chromosome. Am. J. Med. Genet. 76:222–228, 1998. © 1998 Wiley-Liss, Inc.

KEY WORDS: fragile sites; trinucleotide repeats; 11q – syndrome

INTRODUCTION

Terminal deletions of chromosome 11q are rare (<1/100,000 births) but may have severe phenotypic consequences. The severity of the phenotypic change increases with the size of the deletion, although the correlation between the size of the deletion and the appearance of specific phenotypic traits is inconsistent [Penny et al., 1995; Pivnick et al., 1996]. Deletions large enough to include 11q24.1 produce Jacobsen syndrome (JS), a disorder characterized by variable combinations of growth and mental retardation, trigonocephaly, facial and digit anomalies, cardiac defects, thrombocytopenia, and pancytopenia [Fryns et al., 1986; Jacobsen et al., 1973; Penny et al., 1995; Pivnick et al., 1996; Schinzel et al., 1977].

Approximately 70–80% of the known 11q deletion breakpoints cluster in 11q23.3, close to a rare folate-sensitive fragile site, FRA11B [Penny et al., 1995]. Rare folate-sensitive fragile sites are known to be caused by the extensive expansion of (CCG)n repeats and hypermethylation of adjacent CpG islands. It has recently been demonstrated that a (CCG)n repeat in the 5’ untranslated region (UTR) of the CBL2 protooncogene is the FRA11B site [Jones et al., 1994, 1995]. This repeat is present in 11 copies in more than 70% of normal individuals but is found to be expanded to several hundred copies in individuals expressing FRA11B. Further, it has been demonstrated that expansion of the (CCG)n repeat at FRA11B was the likely cause of chromosome 11q deletion in 2 cases of JS [Jones et al., 1994, 1995]. Two families were described in which the mother of a JS patient had an expansion of one copy of the CBL2 (CCG)n repeat. In one family, both the mother and the brother of the patient had an expansion to 750 copies of the (CCG)n repeat and expressed the fragile site. In the second family, the mother had an expansion to 85 copies of the repeat but did not express FRA11B. In both cases, haplotype analysis showed that the deleted chromosome was derived from the maternal chromosome, which carried the expanded repeat, and fluorescence in situ hybridization (FISH) analysis localized the breakpoints to within 20 kb of

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the CBL2 (CCG)n repeat. More recent work has localized these breakpoints to within 10 kb of the CBL2 (CCG)n repeat (E. Baker, unpublished data). In a third family, without a JS patient, the (CCG)n repeat expanded from 80 in the mother to several hundred in each of her 3 children, 1 of whom showed a mosaic expansion and all of whom, unlike the mother, expressed FRA11B. This finding demonstrates that there is a "premutation" level of expansion, which does not trigger expression of FRA11B but is predisposed to marked expansion in future generations. In this respect, the FRA11B site resembles the FRAXA site responsible for the fragile X syndrome, another example in which expansion of a (CCG)n repeat causes expression of a rare folate-sensitive fragile site [Tarleton and Saul, 1993].

If there is an association among FRA11B, an expanded (CCG)n repeat in CBL2, and subsequent del(11)(q23.3), it would be of obvious significance with respect to predicting the risk for JS in families in which FRA11B or the expanded CBL2 (CCG)n repeat is present. We studied 2 JS patients whose deletion breakpoints were within band 11q23.3 to determine the breakpoint and parental origin of the deleted chromosome and to determine whether the deletion was caused by expansion of the CBL2 (CCG)n repeat in the parents.

SUBJECTS AND METHODS

Clinical Data

Patient VH (Fig. 1a) has been described elsewhere [Pivnick et al., 1996]. Briefly, she had the following signs of JS: growth retardation, developmental delay, trigonocephaly with bulging forehead, shallow orbits, hypertelorism, downslanting palpebral fissures, abnormally formed and apparently low-set ears, and an endocardial cushion defect. Anomalies in the central nervous system (CNS) included a small infundibulum and pituitary gland, thickened abnormal gray matter, a deficiency of white matter, progressive ventriculomegaly involving the lateral and third ventricles, and polymicrogyria or pachygyria in the cerebral hemispheres. Other anomalies not usually found in JS patients included hypotonia, central hypothyroidism, moderate bilateral hearing loss, and bilateral inferior colobomas of the irides, which extended to the choroid and retina.

The second patient, AD (Fig. 1b), was born at 40 weeks gestation to a 17-year-old primigravida woman by low forceps vaginal delivery following an uncomplicated pregnancy. The family history was noncontributory. Her birth weight was 3,100 g (50th centile), length was 52 cm (75th centile), and orbito-frontal circumference (OFC) was 34.2 cm (25th centile). Apgar scores were 3, 5, and 7 at 1, 5, and 10 minutes, respectively. She presented with anemia and thrombocytopenia, which were attributed to amnionitis following an 18-hour labor. She developed megalencephaly, and computed tomography showed ventriculomegaly and cortical atrophy at 8 months of age. An ophthalmic examination showed exotropia and hyperopia. On physical examination at 1 year of age, her length was 72 cm (10th centile), weight 7 kg (<5th centile), and OFC 48.2 cm (>95th centile). Her anterior fontanel measured 2 × 3 cm. She had frontal bossing, a depressed nasal bridge, epicanthal folds, a short nose with a flattened

Fig. 1. Patient VH at 2 years of age (a) and patient AD at 6 months of age (b).
nasal tip, downturned mouth with a thin upper lip, overfolding of the superior pinnae, and downslanting of the antihelix. Skin findings included a 2 × 3 cm hypopigmented macule of the abdomen, a mongolian spot on the sacrum, and capillary hemangiomas of the right nostril, nasal tip and midforehead. She had two upper and two lower central incisors. AD had generalized hypotonia and her development was delayed. At age 1 year she could not sit unsupported but reportedly had a vocabulary of 4 words.

AD presented at 1 year of age with RSV pneumonia. A cardiac malformation was identified, and catheterization demonstrated a ventricular septal defect that was obstructed by an aneurysmal tricuspid valve and pulmonary hypertension. She required mechanical ventilation and cardiotropic medication. In addition to the RSV, she had a citrobacter urinary tract infection. She developed bloody diarrhea, and cultures contained Clostridium difficile. She had DIC with thrombocytopenia and anemia requiring blood transfusions. Her status was thought to be improving when she suddenly developed ventricular fibrillation and died.

**Cytogenetic Analyses**

Chromosome analyses of cultured blood lymphocytes were carried out using standard methods. FISH was performed using chromosome-specific paint for chromosome 11, all human telomere probe (Oncor, Inc.), and several locus-specific YAC, PAC, and cosmids probes. YAC clones were generously provided by Dr. Michael James, Oxford University. Cosmid clones were generously provided by Dr. Glen Evans, University of Texas Southwest Medical Center. PAC clones were isolated by the polymerase chain reaction (PCR) from the de Jong PAC library [Ioannou and de Jong, 1996], provided by the MRC HGMPS Resource Center, Cambridge, UK. The order and cytogenetic band localization of the microsatellite markers used are as presented in Gypay et al. [1994], James et al. [1994], and the Genome Database. The marker content of clones used for FISH analysis was obtained from James et al. [1994], Ioannou and de Jong [1996], and unpublished work by M. James (personal communication). FISH analysis with the commercial probes was done according to the manufacturer’s instructions. YACs, PACs, and cosmids were labeled with biotin-11-dUTP using nick translation together with the commercial probes was done according to the manufacturer’s instructions. YACs, PACs, and cosmids were labeled with biotin-11-dUTP using nick translation [Rigby et al., 1977]. Hybridization, washing, and detection were carried out using standard protocols.

**Microsatellite Analysis**

Microsatellite markers were amplified by PCR in a reaction containing 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM MgCl2, 0.67 μM of each primer, 200 μM dATP, dGTP and dTTP, 2.5 μM unlabeled dCTP, 16.7 nM P32-labeled dCTP (3,000 Ci/mmole), and 0.45 units of Amplitaq DNA polymerase (Perkin-Elmer/Cetus), in a total volume of 15 μl. Samples underwent denaturing at 94°C for 5 minutes and then 27 cycles of 94°C for 1 minute, 55°C for 2 minutes, and 72°C for 1 minute, followed by a 6-minute final extension period at 72°C.

The (CCG)n repeat in the 5′ UTR of the CBL2 protooncogene was amplified using primers and a reaction buffer (including deaza-dGTP) that have been described previously [Richards et al., 1996], together with “Red Hot” DNA polymerase (Advanced Biotechnologies). Samples were denatured at 96°C for 2 minutes, followed by 35 cycles at 94°C for 1 minute, 65°C for 1.5 minutes, and 72°C for 1.5 minutes. All PCR products were electrophoresed on a 6% acrylamide/7 M urea gel and visualized by autoradiography with Kodak X-OMAT AR film.

**Southern Blots**

Genomic DNA was extracted from leukocytes using standard techniques. Following the manufacturer’s instructions, 10-μg samples of genomic DNA from each patient were digested in triplicate with 100 units each of XbaI and BglIII, after which one set was digested further with 100 units of the methylation-sensitive restriction enzyme SacII and one set was digested with the methylation-sensitive restriction enzyme SmaI. Digestion products were electrophoresed overnight in 1% agarose gels and blotted onto Zeta Probe GT nylon membranes (Bio-Rad) using alkaline capillary transfer. Probe XS2 is a 1,080-bp fragment that was excised from a pUC18X clone of the 3-kb Sac I fragment from cosmid cA0353 [Jones et al., 1995] by digestion first with XbaI and then with Sac II. The probe was labeled by standard random primer labeling (Boehringer-Mannheim). Prehybridization and hybridization were performed at 65°C for 3 and 16 hours, respectively, in a dextran sulfate/Denhardt’s hybridization solution with 0.5 mg/ml sonicated salmon sperm DNA [Sambrook et al., 1989]. The membranes were washed twice for 15 minutes each time at room temperature in 2× SSC, 0.1% SDS and then for 20 minutes at 65°C in 0.2× SSC, 0.1% SDS. Autoradiography was performed at ~80°C using Kodak X-OMAT AR film.

**RESULTS**

Both patients showed 46,XX,del(11)(q23.3) karyotypes. Microsatellite analysis showed that the deletion was on the paternally derived chromosome in both patients (Table I). The deletion breakpoint for AD was within a 3-cM region, between D11S924 and D11S925. The deletion breakpoint for VH was within a 5-cM region, between D11S924 and D11S1336. Both regions are within band 11q23.3, consistent with the patients’ karyotypes. In addition, the loss of markers D11S969 and D11S968, close to the telomere, suggested that the deletions were terminal rather than interstitial.

VH was studied further using FISH to determine her deletion breakpoint more precisely. FISH analysis with whole chromosome paint showed uniform intensity all along both chromosomes 11. The absence of nonhybridizing areas on either chromosome 11 and the absence of hybridization signal on any other chromosome ruled out the possibility of cryptic rearrangements. Sequential FISH analysis with all-human telomere probe
along with the chromosome 11 alpha-satellite probe showed specific signals at the long-arm telomeres of both chromosomes 11. This finding is consistent with the chromosome deletion having been stabilized by either the addition of a new telomere or telomere capture.

The results of FISH analysis on VH with several locus-specific cosmid, PAC, and YAC probes are summarized in Table II. PAC dJ189-A16, containing the THY1 gene (Fig. 2), and cosmid c11q4b7, containing the CD3 genes [Pivnick et al., 1996], both of which lie centromeric to D11S924, showed specific signals on both chromosomes 11. YAC y969D07 contains the microsatellite marker D11S924, which was shown by PCR to be retained (Table I). However, by FISH analysis, this YAC appeared to be lost from the deleted chromosome because a specific positive signal was seen only on the normal chromosome 11 (Fig. 3). Because D11S924 lies in the centromeric end of YAC y969D07, the breakpoint for VH’s deletion apparently was in the portion of YAC y969D07 that is distal to marker D11S924. The YAC y133B06, which overlaps YAC y969D07 but contains markers distal to D11S924, was also lost from the deleted chromosome. Radiation hybrid mapping data suggest that the distance between D11S924 and the most distal microsatellite marker contained in y969D07, D11S667, is 29.1 cR, representing approximately 1.5 Mb [James et al., 1994]. The FISH and microsatellite findings indicated that VH’s deletion breakpoint was within this 1.5-Mb region. Patient AD’s deletion breakpoint was close to that of VH, in the 62.7-cR (3.1 Mb) region between D11S924, which was retained, and D11S925, which was absent from the paternally derived chromosome 11 (Table I).

There was no evidence of expansion of the (CCG)n repeat in the 5' UTR of the CBL2 protooncogene in either family. Southern blotting showed the same banding pattern in both patients and their parents, as was seen in unaffected control subjects (data not shown). PCR amplification of the CBL2 (CCG)n repeat showed normal-sized alleles in both patients and their parents (Table I). These results demonstrate that the CBL2 (CCG)n repeat is not expanded in these families and that the FRA11B locus is retained on the deleted chromosome of patients AD and VH. We also examined the CBL2 repeat lengths in 4 patients reported by Penny et al. [1995] whose deletion breakpoint intervals overlapped with the intervals described here (Fig. 3). Jones et al. [1995] found that these deletions were not caused by FRA11B because Southern analysis showed no evidence of CBL2 repeat expansions in the parents of these patients. More recently, PCR analysis has revealed that the CBL2 repeat lengths were 11, 11, 11/13, and 11/14 in patients 4, 5, 6, and 7, respectively. In each case, these CBL2 alleles were consistent with direct inheritance from their parents (data not shown). This further demonstrates that FRA11B was not responsible for the chromosome deletion in patients 6 and 7 and that the deletion breakpoints in these 2 patients were telomeric to FRA11B. These data suggest that the deletion breakpoints for patients 6 and 7 lie within overlapping regions spanning 93.2 cR/4.7 Mb and 184.6 cR/9.3 Mb, respectively.

### DISCUSSION

A combination of microsatellite and FISH analyses has localized the 11q deletion breakpoints of 2 JS patients to within two short overlapping intervals (approximately 1.5 and 3.1 Mb) within band 11q23.3.

<table>
<thead>
<tr>
<th>TABLE II. FISH Analysis on VH*</th>
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<tr>
<td>FISH probe</td>
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<tr>
<td>c11q4b7</td>
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<tr>
<td>dJ44-N2</td>
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<tr>
<td>dJ61-11</td>
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<tr>
<td>dJ189-A16</td>
</tr>
<tr>
<td>y969D07</td>
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<td>y133B06</td>
</tr>
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</table>

*The marker content of the mapping reagents used was obtained from James et al. [1994], Ioannou and de Jong [1996], or unpublished work by M. James (personal communication).

bProbes in 11q23.3 are in proximal-distal order as specified by James et al. [1994] and Gyapay et al. [1994].

c++, Probe present on both chromosomes 11; +, probe present on the normal chromosome 11 but not on the deleted chromosome 11.

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*The order and band localization of the microsatellite markers are as presented by James et al. [1994], Gyapay et al. [1994], and in the Genome Database. Markers that were unequivocally deleted are indicated in bold type. Numbers have been arbitrarily assigned, with the lowest number representing the largest dinucleotide repeat. The order and band localization of the microsatellite markers are as presented by James et al. [1994], Gyapay et al. [1994], and in the Genome Database.

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**TABLE I. Microsatellite Analysis**

<table>
<thead>
<tr>
<th>Band Marker</th>
<th>Mother VH</th>
<th>Father</th>
<th>Family 1</th>
<th>Mother AD</th>
<th>Father</th>
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<tr>
<td>q23.3 D11S976</td>
<td>1.1</td>
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<td>2.3</td>
<td>1.2</td>
<td>2.3</td>
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<tr>
<td>q23.3 CBL2</td>
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<td>11.12</td>
<td>11</td>
<td>12.17</td>
<td>8.12</td>
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<tr>
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<td>1.2</td>
<td>1.1</td>
<td>2.3</td>
<td>1.2</td>
</tr>
<tr>
<td>q23.3 D11S925</td>
<td>2.2</td>
<td>2</td>
<td>1.2</td>
<td>1.3</td>
<td>1</td>
</tr>
<tr>
<td>q23.3-24.3 D11S1336</td>
<td>1.3</td>
<td>3</td>
<td>2.4</td>
<td>1.2</td>
<td>2</td>
</tr>
<tr>
<td>q23.3-24.2 D11S1353</td>
<td>2.3</td>
<td>3</td>
<td>1.1</td>
<td>1.2</td>
<td>2</td>
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<tr>
<td>q23.3-24 D11S934</td>
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<td>1.3</td>
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<tr>
<td>q24 D11S910</td>
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<td>Not tested</td>
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<td>0.3</td>
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<tr>
<td>q24 D11S439</td>
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<td>4</td>
<td>1.3</td>
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<td>1</td>
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<tr>
<td>q23.2-24 D11S836</td>
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<td>2</td>
<td>1.3</td>
<td>1.2</td>
<td>1</td>
</tr>
<tr>
<td>q24-pter D11S874</td>
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<td>3</td>
<td>1.3</td>
<td>3.4</td>
<td>4</td>
</tr>
<tr>
<td>q24.1-q25 D11S969</td>
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<td>2</td>
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<td>1.2</td>
<td>2</td>
</tr>
<tr>
<td>q24.1-q25 D11S968</td>
<td>2.3</td>
<td>2</td>
<td>1.2</td>
<td>Not tested</td>
<td>1.2</td>
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</table>
CCG repeats have been found within a 2-Mb region that would not be unique in the human genome. Seven Mb of FRA11B. Such an arrangement of fragile sites may lead to the identification of new genes involved in JS. In addition, there is a common aphidicolin-inducible fragile site in band 11q23.3 (FRA11G), the location and molecular mechanism of which are presently unknown.

It is of interest to note that the deleted chromosomes in both patients were paternal in origin, whereas the 2 deletions caused by expansion of the FRA11B (CCG)n repeat were maternal in origin. The study by Penny et al. [1995] showed a bias toward paternally derived deletions in cases where the breakpoint was distal to the D11S924 marker. Five of 6 patients with deletion breakpoints distal to D11S924 and 2 others whose breakpoints may be distal to D11S924 had deletions on the paternally derived chromosome. The addition of 2 more paternally derived deletions with breakpoints distal to D11S924 makes it increasingly unlikely that this bias is due to chance alone. In contrast, of the 8 deletions with breakpoints centromeric to D11S924, only 3 were paternal in origin, whereas 5 were maternal in origin, including 2 maternally derived deletions caused by FRA11B. Several clinically significant trinucleotide repeats show different propensities for expansion when transmitted through the maternal versus paternal germline [Fu et al., 1991; Lavedan et al, 1993; Longshore and Tarleton, 1996; Yu et al., 1991]. FRA11B shares several other characteristics with other clinically significant trinucleotide repeats, including the existence of a premutation expansion range and the ability for the repeat expansion to contract when passed through one of the parental germlines [Jones et al., 1995; Lavedan et al., 1993; Rousseau et al., 1995]. More work is clearly needed to characterize the effect that parent of origin exerts on the propensity for trinucleotide repeat lengths to change and on the specific sites at which chromosomes break.

Full expression of JS appears to involve a number of genes in the distal 11q region. It has already been noted that the ETS1 and NFRKB genes encode proteins that interact with several genes in hematopoietic cells and may be responsible for the thrombocytopenia and pancytopenia seen in many JS patients [Penny et al., 1995]. The FLI1 gene, just distal to the ETS1 gene, is also expressed in hematopoietic cells and could also be involved in these manifestations. The THY1 gene, located just distal to CBL2 in 11q23.3, encodes a T-cell surface glycoprotein, is expressed at significant levels in fibroblasts, and stimulates neuronal growth and development. These pleiotropic effects could contribute to the immune deficiency, craniosynostosis, and CNS malformations seen in JS patients. However, because the THY1 gene was not deleted in either JS patient, our findings suggest that, despite these possibilities, the THY1 gene is not a primary contributor to the JS phenotype. One gene in this region that could contribute to the developmental anomalies seen in JS but has not yet been suggested as a candidate gene is the signal recognition particle receptor gene in 11q24.1. This gene encodes the "docking protein" that constitutes the first step in the sequence of events by which newly synthesized proteins are transported across the rough endoplasmic reticulum membrane and enter the secretory pathway [Hortsch et al., 1988].

### Table: Deletion Intervals of Patients with Jacobsen Syndrome

<table>
<thead>
<tr>
<th>#4</th>
<th>#5</th>
<th>#6</th>
<th>#7</th>
<th>AD</th>
<th>VH</th>
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</thead>
<tbody>
<tr>
<td>cR</td>
<td>Mb</td>
<td>cR</td>
<td>Mb</td>
<td></td>
<td></td>
</tr>
<tr>
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<td>1.08</td>
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<tr>
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<td>1.53</td>
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<td></td>
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<tr>
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<td>1.46</td>
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<tr>
<td>D11S925</td>
<td>33.6</td>
<td>1.69</td>
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<tr>
<td>D11S667</td>
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<td>1.16</td>
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<tr>
<td>D11S1336</td>
<td>2.86</td>
<td>1.3</td>
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</table>

Fig. 2. Deletion intervals of patients with Jacobsen syndrome described in the text (patients AD and VH) and by Penny et al. [1995] (#4–#7). White bars represent material retained and black bars material lost. The striped bars indicate the possible location of the deletion breakpoint in each patient. The distances between the markers are shown in cR (left) and Mb (right), according to the radiation hybrid map, at an estimated 1 cR0.001 = 50.2 kb [James et al., 1994].

However, unlike the 2 patients reported previously [Jones et al., 1994, 1995], neither of these deletions appears to be due to expansion of the (CCG)n repeat in the CBL2 gene and expression of the FRA11B site. The breakpoint regions in both these patients lie telomeric to the FRA11B site. Further, they overlap the breakpoint regions in 4 patients whose breakpoint regions were originally reported to include FRA11B but whose deletions have also since been found not to be due to expansion of the CBL2 (CCG)n repeat and expression of FRA11B [Jones et al., 1994, 1995]. Although FRA11B is the only folate-sensitive fragile site in 11q23.3 to have been described to date, the presence of an additional, previously uncharacterized, folate-sensitive fragile site in this region should not be ruled out. For example, although this is purely speculative, the 6 deletions depicted in Figure 2 could be accounted for by a second CCG repeat/folate-sensitive fragile site within 3 Mb of FRA11B. Such an arrangement of fragile sites would not be unique in the human genome. Seven (CCG)n repeats have been found within a 2-Mb region of chromosome 4 [Hummerich et al., 1994] and 3 folate-sensitive fragile sites have been mapped within 1–2 Mb on the X chromosome [Willems, 1994]. Because all folate-sensitive fragile sites known to date are associated with CpG islands, discovery of additional trinucleotide-related fragile sites in this region of chromosome 11 may lead to the identification of new genes involved in JS. In addition, there is a common aphidicolin-inducible fragile site in band 11q23.3 (FRA11G), the location and molecular mechanism of which are presently unknown.

It is of interest to note that the deleted chromosomes in both patients were paternal in origin, whereas the 2 deletions caused by expansion of the FRA11B (CCG)n repeat were maternal in origin. The study by Penny et al. [1995] showed a bias toward paternally derived deletions in cases where the breakpoint was distal to the D11S924 marker. Five of 6 patients with deletion breakpoints distal to D11S924 and 2 others whose breakpoints may be distal to D11S924 had deletions on the paternally derived chromosome. The addition of 2 more paternally derived deletions with breakpoints distal to D11S924 makes it increasingly unlikely that this bias is due to chance alone. In contrast, of the 8 deletions with breakpoints centromeric to D11S924, only 3 were paternal in origin, whereas 5 were maternal in origin, including 2 maternally derived deletions caused by FRA11B. Several clinically significant trinucleotide repeats show different propensities for expansion when transmitted through the maternal versus paternal germline [Fu et al., 1991; Lavedan et al, 1993; Longshore and Tarleton, 1996; Yu et al., 1991]. FRA11B shares several other characteristics with other clinically significant trinucleotide repeats, including the existence of a premutation expansion range and the ability for the repeat expansion to contract when passed through one of the parental germlines [Jones et al., 1995; Lavedan et al., 1993; Rousseau et al., 1995]. More work is clearly needed to characterize the effect that parent of origin exerts on the propensity for trinucleotide repeat lengths to change and on the specific sites at which chromosomes break.

Full expression of JS appears to involve a number of genes in the distal 11q region. It has already been noted that the ETS1 and NFRKB genes encode proteins that interact with several genes in hematopoietic cells and may be responsible for the thrombocytopenia and pancytopenia seen in many JS patients [Penny et al., 1995]. The FLI1 gene, just distal to the ETS1 gene, is also expressed in hematopoietic cells and could also be involved in these manifestations. The THY1 gene, located just distal to CBL2 in 11q23.3, encodes a T-cell surface glycoprotein, is expressed at significant levels in fibroblasts, and stimulates neuronal growth and development. These pleiotropic effects could contribute to the immune deficiency, craniosynostosis, and CNS malformations seen in JS patients. However, because the THY1 gene was not deleted in either JS patient, our findings suggest that, despite these possibilities, the THY1 gene is not a primary contributor to the JS phenotype. One gene in this region that could contribute to the developmental anomalies seen in JS but has not yet been suggested as a candidate gene is the signal recognition particle receptor gene in 11q24.1. This gene encodes the "docking protein" that constitutes the first step in the sequence of events by which newly synthesized proteins are transported across the rough endoplasmic reticulum membrane and enter the secretory pathway [Hortsch et al., 1988]. Such a protein is im-
important in the proper development and functioning of many cell types and could contribute to the phenotype involved in JS and a wide variety of other genetic disorders.

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Fig. 3. FISH results using YAC y969D07 (upper left) and PAC dJ189-A16 (upper right). The corresponding G-banded karyotypes are pictured below the FISH images. For y969D07, no signal is present on the deleted chromosome 11 (green arrowhead), whereas a signal is clearly present on the nondeleted chromosome 11 (blue arrowhead). For dJ189-A16, signals are clearly present on both chromosomes 11.


