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Molecular Analysis of Deletion (17)(p11.2p11.2) in a Family Segregating a 17p Paracentric Inversion: Implications for Carriers of Paracentric Inversions

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Summary

A male child with multiple congenital anomalies initially was clinically diagnosed as having Smith-Lemli-Opitz syndrome (SLOS). Subsequent cytogenetic studies revealed an interstitial deletion of 17p11.2, which is associated with Smith-Magenis syndrome (SMS). Biochemical studies were not supportive of a diagnosis of SLOS, and the child did not display the typical SMS phenotype. The father's karyotype showed a paracentric inversion of 17p, with breakpoints in p11.2 and p13.3, and the same inversion was also found in two of the father's sisters. FISH analyses of the deleted and inverted 17p chromosomes indicated that the deletion was similar to that typically seen in SMS patients and was found to bracket the proximal inversion breakpoint. Available family members were genotyped at 33 polymorphic DNA loci in 17p. These studies determined that the deletion was of paternal origin and that the inversion was of grandpaternal origin. Haplotype analysis demonstrated that the 17p11.2 deletion arose following a recombination event involving the father's normal and inverted chromosome 17 homologues. A mechanism is proposed to explain the simultaneous deletion and apparent "reinversion" of the recombinant paternal chromosome. These findings have implications for prenatal counseling of carriers of paracentric inversions, who typically are considered to bear minimal reproductive risk.

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Introduction

Chromosomal inversions occur when a single chromosome breaks at two different sites, followed by a 180° rotation and reunion of the intrachromosomal segment. Pericentric inversions (PEIs) occur when one break occurs in the short arm and the other in the long arm, whereas paracentric inversions (PAIs) occur when both the breaks and the reunion occur within the same chromosome arm. Chromosomal inversions, in general, are not infrequent, with an average incidence of 0.13 per 1,000 live borns. Although the true incidence of PAIs is unknown, estimates of prevalence of PAIs range from 0.09 per 1,000 live borns to 0.49 per 1,000 live borns (Pettenati et al. 1995). Because banding patterns may change little, PAIs may be more difficult to detect than PEIs. There usually is no phenotypic effect in the majority of inversion carriers. However, when an inversion disrupts a critical gene, an associated disease phenotype can result (Pettenati et al. 1995).

The outcome of meiotic recombination that involves the inverted chromosomal regions in heterozygote carriers of a PAI can result in a dicentric chromatid, an acentric chromatid, a normal chromatid, or a chromatid with a PAI (Therman 1980). Zygotes that bear the dicentric or the acentric chromatids are assumed to be inviable, and thus the reproductive risk for carriers is assumed to be minimal (Vogel and Motulsky 1986; Gardner and Sutherland 1989).

A review of 446 cases of PAI identified inversions in all chromosomes, with chromosomes 1, 3, 5, 6, 7, 11, and 14 most frequently involved (Pettenati et al. 1995). Of those PAIs, 66.6% were inherited, 8.5% arose de novo, and the rest were of undetermined origin. A strong association between PAIs and mental retardation/multiple congenital anomalies (MR/MCA) was observed, which is not unexpected given that cytogenetic evaluation had likely been conducted to investigate the etiology of MR/MCA in these cases. De novo PAIs were more likely to be associated with MR/MCA (31.6%) than were inherited PAIs (22.0%). Viable offspring with recombinant chromosomes occurred in 17 (3.8%) of the

446 PAI cases. These included two dicentrics, eight duplications, six deletions, and one case with a duplication/deletion. All but one of the viable recombinant offspring had inherited their PAI. To date, no detailed molecular analysis has been conducted on such viable recombinant offspring. Molecular analysis would allow examination of the various models proposed to account for recombinations involving PAIs that result in chromosomal aberrations. Determination of the origin of such recombinants at the molecular level would also allow critical evaluation of reproductive risk, for carriers of PAIs.

We report a child with multiple congenital anomalies, who was clinically diagnosed initially as having Smith-Lemli-Opitz syndrome (SLOS; MIM 270400) (Smith et al. 1964; McKusick 1994), which has been recognized as an important and possibly frequent MR/MCA syndrome. The major manifestations of SLOS include prenatal and postnatal growth retardation, microcephaly, characteristic facial appearance, syndactyly of toes 2 and 3, and genital anomalies. Some clinical researchers have postulated the existence of a type II SLOS (MIM 268670) (McKusick 1994) on the basis of more severe clinical manifestations, such as early lethality, postaxial polydactyly, and male pseudohermaphroditism. Cases of type I and type II SLOS are known to occur in the same sibship, and a low serum-cholesterol level is present in both types (Tint et al. 1995). On the basis of reports of SLOS patients carrying chromosomal aberrations, it has been proposed that there is a causative gene located at 7q32 (Berry et al. 1989; Opitz 1994; Wallace et al. 1994).

The proband in our study died at 2 mo of age. Subsequent biochemical studies revealed that he had below-normal cholesterol levels, but 7-dehydrocholesterol was undetectable, which ruled out SLOS. However, cytogenetic studies revealed an interstitial deletion of 17p11.2, a common cytogenetic abnormality associated with Smith-Magenis syndrome (SMS; MIM 182290) (McKusick 1994). SMS is an MR/MCA syndrome with characteristic behavioral and physical phenotypic features, which are noted in the clinical diagnosis of the syndrome in children and adults. These features include moderate to severe mental retardation, self-injurious behavior, sleep disturbances, brachycephaly, broad face, flat midface, myopia, strabismus, short broad hands, and syndactyly of toes 2 and 3 (Smith et al. 1986; Stratton et al. 1986; Colley et al. 1990; Allen et al. 1991; Greenberg et al. 1996). Whereas the majority of patients demonstrate a common deletion interval within 17p11.2 (Juyal et al. 1996), deletions can range in size from <2 Mb to 9–10 Mb (Trask et al. 1996).

The phenotypically normal father of the proband was shown to have a PAI of 17p. This inversion was also evident in two of his phenotypically normal sisters. We investigated the origin of the 17p11.2 deletion in the proband by constructing haplotypes of polymorphic markers spanning and flanking the relevant deleted and inverted segments on the short arm of chromosome 17, in the proband and in his family members. Our analysis supports the hypothesis that the 17p11.2 deletion occurred as a result of a recombination between the normal and the paracentric-inverted chromosome 17 homologues of the proband's father.

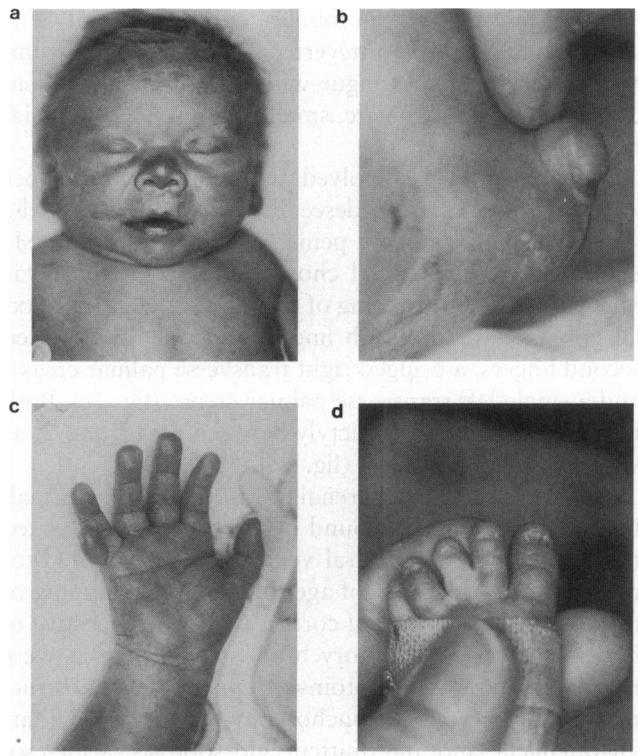

Figure 1 Phenotypic features of proband, PAT42, at 2 d of age. *a*, Frontal view showing dysmorphic craniofacial features. *b*, Male genitalia. *c*, Hand showing polydactyly, long fifth finger, and radially deviated second finger. *d*, Foot showing cutaneous syndactyly between toes 2 and 3.

Subject and Methods

Subject

The male propositus (PAT42) was born to a 28-year-old woman with a history of one previous spontaneous abortion. Pregnancy was complicated by polyhydramnios and by a poor biophysical profile, in the third trimester. Because of fetal distress, an operative delivery was performed, and birth weight (2,170 gm), length (44 cm), and head circumference (31.5 cm) all were appropriate for the gestational age of 35.5 wk. Physical exam at 2 d of age showed generalized edema, especially over the posterior scalp and neck. The following dysmorphic craniofacial features were noted (fig. 1*a*): anteriorly displaced parietal hair whorl, cup-shaped ears with thick-

ened helices and uplifted lobules, epicanthal folds, left-sided iris coloboma, anteverted nares, long philtrum, thin upper lip, small tongue with prominent sublingual tissue, high narrow palate, small jaw, and flat midfacial hemangioma.

Major anomalies involved the genitalia and upper limbs. The testes were descended incompletely in the inguinal canals, and the penis was small (1 cm; 6 SD < mean), with a ventral chordee (fig. 1*b*). There was mild rhizomelic shortening of the arms, bilateral postaxial polydactyly, long fifth fingers and radially deviated second fingers, a bridged right transverse palmar crease, and a single left transverse palmar crease (fig. 1*c*). Both feet had cutaneous syndactyly between toes 2 and 3, as well as hypoplastic nails (fig. 1*d*).

Results of cardiac and renal ultrasounds were normal, but results of the ultrasound of the head demonstrated mild dilation of the lateral ventricles. A computed-tomography scan at 2 wk of age showed focal thinning of the right parieto-occipital cortex, which is suggestive of ischemic changes. Auditory brain-stem responses were normal bilaterally. Symptoms of upper-airway obstruction led to fiberoptic bronchoscopy, which showed laryngomalacia and intermittent glossoptosis. Cine-esophagography confirmed poor coordination of sucking, swallowing, and breathing.

The results of routine blood chemistries were typical of liver disease—aspartate aminotransferase activity of 1,050 IU/liter (normal 0–40 IU/liter), alanine aminotransferase activity of 940 IU/liter (normal 0–45 IU/liter), 2.5 g albumin/dl (normal 3.0–5.5 g/dl), and 10.1 ng alpha-fetoprotein/ml (normal 0–8.5 ng/ml). The results of an endocrine workup demonstrated impaired testicular function: baseline testosterone was 2.2 ng/dl (normal 20–50 ng/dl), increasing to only 4.8 ng/dl after 5 d of human chorionic gonadotropin (hCG) injections. Cortisol was 20.3 μg/dl at 8 A.M., indicating normal adrenal function. The calcium level of 7.4 mg/dl (normal 8.4–10.5 mg/dl) and the phosphorus level of 3.0 mg/dl (normal 5.8–9.0 mg/dl) were suggestive of vitamin D deficiency, but vitamin D metabolites were never measured directly. Total cholesterol was only 51 mg/dl (normal 65–175 mg/dl). 7-Dehydrocholesterol accumulation, typically seen in SLOS, did not occur in blood or in cultured fibroblasts (R. I. Kelley, personal communication). Results of special tests for red-blood-cell plasmalogens and plasma very-long-chain fatty acids were normal. Essential-fatty-acid deficiency was suspected on the basis of a mild elevation of the triene/tetraene ratio to 0.076 (normal = 0.02), even though the blood was drawn at 3 d of age (A. B. Moser, personal communication).

The propositus died at age 2 mo, and no autopsy was done. The next pregnancy resulted in the birth of a healthy female, after prenatal diagnosis, by amniocentesis, of a balanced inversion.

Establishment of Cell Lines and DNA Isolation

A skin biopsy of the propositus, PAT42, was performed and a fibroblast cell line was established by use of standard procedures. Blood samples were collected from the proband's parents (PAT43 and PAT44), to establish lymphoblastoid cell lines (Anderson and Gusella 1984) and to isolate genomic DNA. Other relatives of the proband, including his paternal great-grandmother (PAT49), grandmother (PAT48), two aunts (PAT45 and PAT46), and uncle (PAT47), were also sampled for DNA isolation. Genomic DNA was isolated by SDS lysis, proteinase K digestion, phenol/chloroform extraction, and ethanol precipitation (Sambrook et al. 1989).

Molecular Analysis

Southern blot analysis was conducted as described in a previous study (Patel et al. 1990), by use of the following 13 polymorphic chromosome 17p markers: EW301, EW401, EW402, EW403, EW405, EW505, VAW409R1, VAW409R3, VAW410, VAW411R2, VAW412R3, and pβ8-2 (Wright et al. 1990) and pYNM67-R5 (Ray et al. 1990). Genotyping at 20 PCR-based hypervariable microsatellite loci was conducted as described in a previous study (Figuera et al. 1995). These loci were as follows (in physical and/or genetic order): D17S520, CHRNB1, D17S922, D17S921, D17S839, D17S122, D17S793, D17S955, D17S953, D17S71, D17S805, D17S446, D17S783, D17S959, D17S842, D17S925, D17S935, D17S841, D17S1294, and D17S798 (Lupski et al. 1991; Guzzetta et al. 1992; Dib et al. 1996).

FISH Analysis

Chromosome preparations were made from lymphocytes or fibroblasts of the propositus and from lymphocytes or Epstein-Barr virus–transformed lymphoblasts of the father by use of standard cytogenetic techniques. The probes used were as follows: cosmids c49E4, c142F7, c94A12, and cH3, representing the locus for the peripheral myelin protein 22 gene (*PMP22*) and the anonymous loci D17S258, D17S29, and D17S446, respectively (Juyal et al. 1996). Cosmids cCI17-498 and cCI17-638 represented anonymous loci in distal 17p11.2 and were obtained from the Japanese Cancer Research Resources Bank (Inazawa et al. 1993). A control cosmid from the thymidine kinase locus in 17q was included in all hybridizations with each of the latter probes (Juyal et al. 1996). A cosmid probe for the Miller-Dieker syndrome locus in 17p13, together with a control cosmid from the retinoic acid–receptor alpha (*RARA*) locus in 17q21.1, was purchased from Oncor®. Cosmid probes were labeled with biotin or with digoxigenin, and FISH analysis was conducted as described in a previous study (Juyal et al. 1996). At least 20 complete metaphase plates were scored for each hybridization.

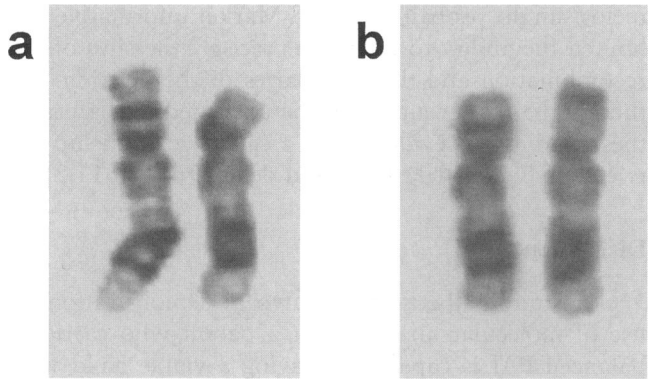

Figure 2 *a*, G-banded chromosome 17 homologues of patient PAT42, with the normal chromosome 17 on the left and del(17)(p11.2p11.2) on the right. *b*, G-banded chromosome 17 homologues of individual PAT44, the father of patient PAT42, with the normal chromosome 17 on the left and inv(17)(p11.2p13.3) on the right.

Results

Cytogenetic Studies

Chromosome analyses by routine G-banding on blood lymphocytes and skin fibroblasts, from the propositus (fig. 2*a*), revealed a karyotype of 46,XY, del(17)(p11.2p11.2). The mother, paternal grandmother, and paternal great-grandmother had normal chromosomes. Cytogenetic studies on the father (fig. 2*b*) revealed what appeared to be a PAI within 17p, with breakpoints at p11.2 and p13. Chromosomes from the father's siblings were also analyzed, and it was found that his two sisters carried the same structural aberration, 46,XX,inv(17)(p11.2p13).

FISH Analysis

FISH analysis using the cosmid cH3 representing the locus D17S446 confirmed the cytogenetically demonstrated interstitial deletion at 17p11.2 in the propositus, PAT42. Additional FISH studies with probes for the loci D17S29 and D17S258, which lie distal to D17S446, confirmed that the deletion in the propositus corresponded to the SMS common deletion interval (Juyal et al. 1996). There was no deletion or rearrangement of the more distal Miller-Dieker and *PMP22* probes, on either chromosome 17 homologue.

The PAI in the proband's father, PAT45, was also confirmed by FISH analysis, and the cytogenetically assigned breakpoint at 17p13 was refined to 17p13.3, by use of the Miller-Dieker probe. The proximal inversion breakpoint within 17p11.2 was determined, by FISH analysis, to lie between *PMP22* and the distal end of the SMS common deletion interval. The other paternal chromosome 17 did not show any alteration with any of the probes tested. Representative results of these FISH studies are shown in figures 3*a* and 3*b*.

A summary of the data obtained by use of all the probes is shown in figure 4. Analyses of these cumulative data indicate that the deletion is similar to that found in the majority of SMS patients. Furthermore, the deletion interval in the proband brackets the proximal end of the inversion in his father.

Genotype and Haplotype Analysis

In order to determine the parental origin of the deletion, genotypes at multiple polymorphic loci spanning 17p were determined. Haplotypes were constructed on the basis of the observed segregation of alleles at 13 partially or completely informative microsatellite-marker loci (fig. 5). The markers are ordered on the basis of the most current version of the Généthon human genetic-linkage map (Dib et al. 1996), as well as by deletion interval–mapping studies in patients with SMS (Juyal et al. 1996). Segregation analysis of marker D17S446 clearly demonstrates lack of a paternal allele in the propositus, PAT42, indicating that the deletion was derived from the father, PAT44.

The origin of the PAI was also deduced via haplotype analysis. The three individuals (PAT44, PAT45, and PAT46) carrying the inversion share the same markers

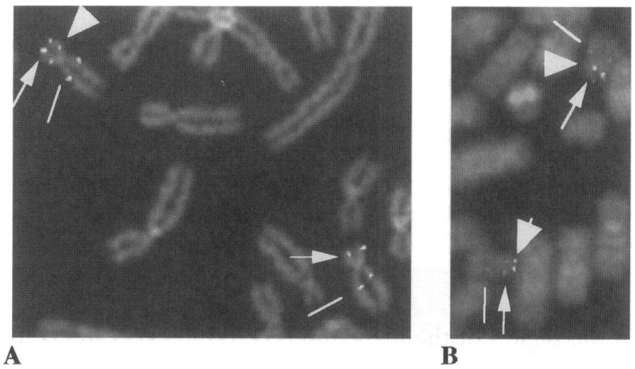

Figure 3 FISH analyses of del(17)(p11.2p11.2) in the proband, PAT42, and inv(17)(p11.2p13.3) in the proband's father, PAT44. *A*, Image of a partial metaphase from patient PAT42, hybridized in situ with cosmid c142F7 (locus D17S258), which maps within the 17p11.2, SMS, region (*arrowhead*); a cosmid from the Miller-Dieker syndrome region (Oncor®) (*arrow*); and a control cosmid from the *RARA* locus on chromosome 17q21.1 (Oncor®) (*bar*). The del(17)(p11.2p11.2) homologue, showing a signal for the Miller-Dieker probe in the normal 17pter location, as well as the signal for the control probe on 17q, but no signal in the 17p11.2 region, is shown (*top*). *B*, Image of a partial metaphase from PAT44, the father of patient PAT42, hybridized in situ with cosmid cCI17-498, which maps within the distal end of 17p11.2 and within the SMS common deletion region (*arrowhead*); a cosmid from the Miller-Dieker syndrome region (Oncor®) (*arrow*); and a control cosmid from the *RARA* locus on chromosome 17q21.1 (Oncor®) (*bar*). On the normal chromosome 17 homologue, the signal corresponding to the Miller-Dieker locus probe is telomeric, and the signal corresponding to the distal SMS interval is centromeric (*top*). On the inv(17)(p11.2p13.3) chromosome (*bottom*), the order of these signals is reversed.

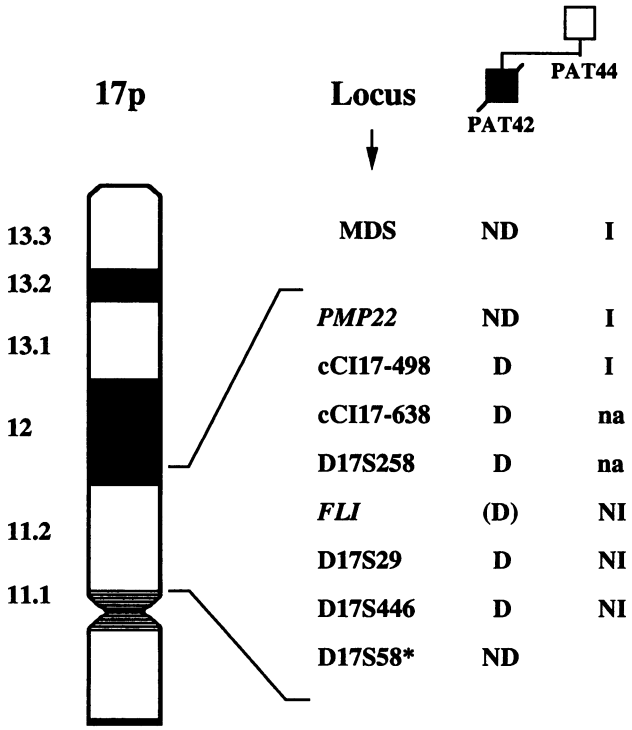

Figure 4 Ideogram of chromosome 17p, schematically illustrating the results of FISH studies on patient PAT42 and his father, PAT44. All loci indicated are within 17p11.2, with the exception of D17S58, which is in 17p11.1, and the Miller-Dieker syndrome locus (MDS), which is in 17p13. "D" and "ND" indicate whether the locus is deleted or not deleted, respectively, on the del(17)(p11.2p11.2) chromosome in PAT42. "(D)" indicates that deletion of the locus is inferred. "I" and "NI" indicate whether the locus is inverted or not inverted, respectively, on the inv(17)(p11.2p13.3) chromosome in PAT44. "na" indicates that the inversion status for these loci was not assessed. The asterisk (*) indicates that the deletion status at this locus was determined by polymorphism analysis.

in the region of the inversion (D17S520–D17S839). By inference, these inversions were inherited from their deceased father, since they do not appear on the haplotypes for their mother, PAT48. A recombination event in the vicinity of marker D17S71 took place when the inverted chromosome 17 was transmitted to PAT44 and PAT45 but not when it was transmitted to PAT46.

The haplotype of the deleted chromosome 17 in PAT42 suggests that it arose from a similar recombination between the inverted grandpaternal and the noninverted grandmaternal chromosomes. The deleted haplotype thus consists of the grandpaternal alleles centromerically and the grandmaternal alleles telomerically. The site of recombination could not be mapped accurately, but the data are consistent with a location between D17S921 and D17S959, which encompasses the proximal inversion breakpoint.

Our data are consistent with the occurrence of a recombination, as well as an interstitial deletion, during meiosis in the proband's father. Marker informativeness limited the ability to delineate precisely the sites of the recombination and the boundaries of the deletion. Although the two events might be unrelated, we interpret these findings as evidence for a causal relationship between inv(17)(p11.2p13.3) and del(17)(p11.2p11.2).

Discussion

Our study is the first unequivocal demonstration by use of molecular analysis that a parent who carries a balanced PAI is capable of having a viable child with an unbalanced monocentric recombinant chromosome. FISH analysis irrefutably documents that the father carries a true PAI, and haplotype analysis supports unequal crossing-over at the base of the inversion loop as the underlying mechanism for the proband's interstitial deletion. Theoretically, the meiotic products resulting from a crossover within the inversion loop are a dicentric chromatid and an acentric fragment, but other possible products can be expected, depending on the number of crossovers, their location, and the involved chromatids (Therman 1980). Pettenati et al. (1995) recently stated in their review of 446 PAI cases that 3.8% were associated with viable recombinant offspring, but reinterpretation of their data to include only the inherited inversions resulted in a figure of 5.4% (16 of 297 cases), with only 2 cases being dicentric and the rest being monocentric (8 duplications, 5 deletions, and 1 combined duplication/deletion), including the family that we are now reporting on in greater detail. Thus, it does appear that interstitial duplications or deletions are fairly common recombination products of a PAI, although the aforementioned frequencies, which are a combined risk for all possible mechanisms, may be biased by the ascertainment of cases with abnormalities. An accurate recurrence risk cannot be inferred from the literature.

We considered several mechanisms that could produce a monocentric recombinant, but these mechanisms all predict the deletion of a much larger portion of an inverted region (under the assumption that there is no simultaneous duplication), limit the deletion to loci within the inversion (under the assumption that no "reinversion" takes place), or produce a terminal rather than an interstitial deletion. The mechanism that best fits all the observations in our case is unequal crossing-over at the base of the meiotic inversion loop, as initially proposed by Hoo et al. (1982). This model completely explains the findings seen in our patient (fig. 6)—that the deletion occurred at the proximal end of the inversion and included loci on both sides of the inversion breakpoint. When unequal crossovers occur at the base of an inversion loop, the resulting duplication or deletion is likely to be small and also is likely to include sequences that lie just outside the inverted region, thus

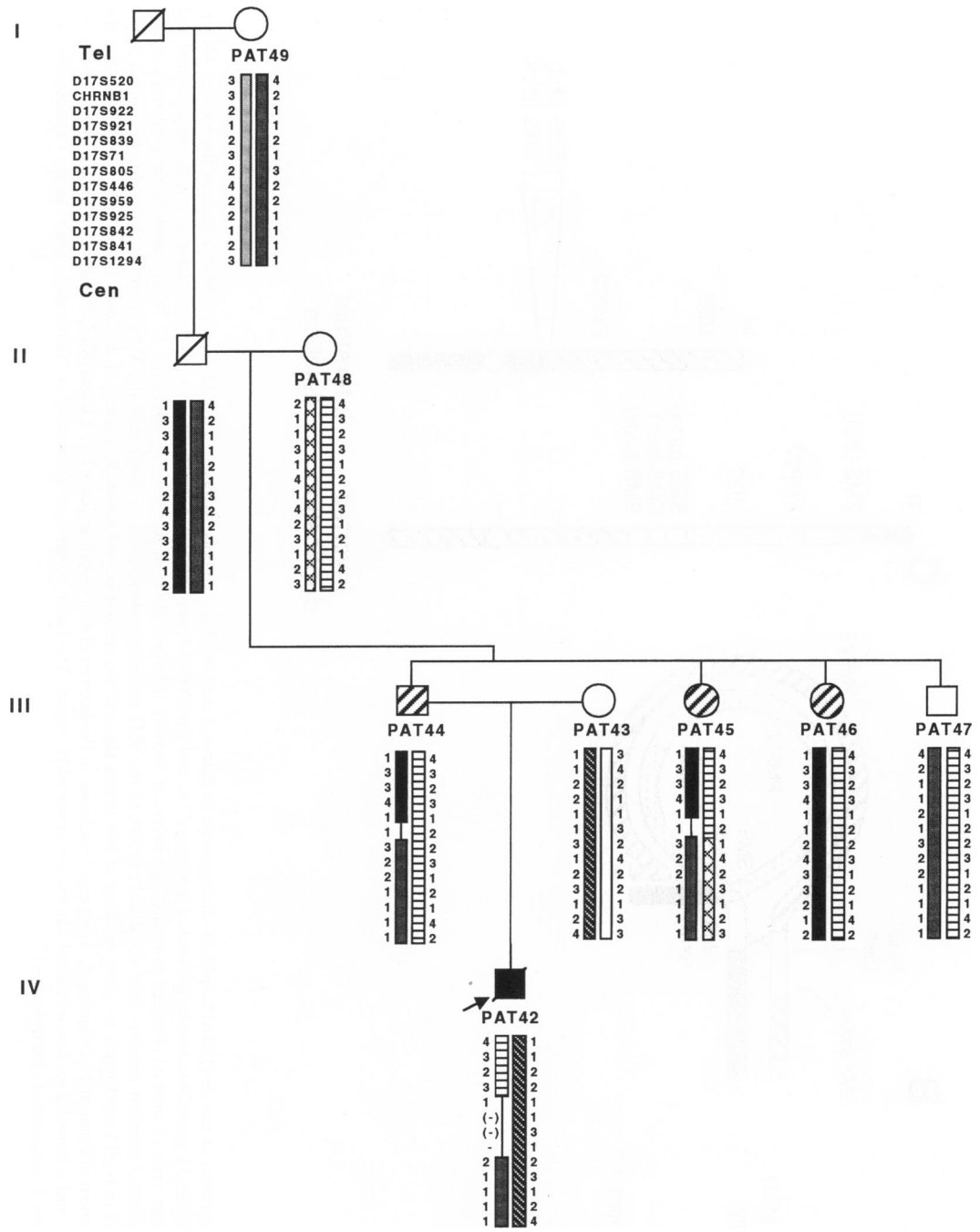

Figure 5 Pedigree showing the segregation of the 17p PAI and haplotypes, at the indicated 17p loci, for family members of patient PAT42. Squares denote males, and circles denote females. The blackened square indicated by an arrow identifies the proband, PAT42, whose karyotype was 46,XY,del(17)(p11.2p11.2) and was indicative of SMS. Hatched circles and squares denote carriers of inv(17)(p11.2p13.3). The loci are ordered on the basis of the genetic map determined at Généthon, in concert with the physical order of markers determined by deletion breakpoints in SMS patients and with a chromosome 17 hybrid panel. Note the absence of a paternal allele at loci D17S953 and D17S446, in PAT42. The grandmaternal and grandpaternal haplotypes of PAT42 are represented by boxes with horizontal lines and gray shading, respectively, and highlight a recombination event on the del(17)(p11.2p11.2) chromosome in PAT42, which is derived from his father. Thin vertical lines in place of boxes (PAT44, PAT45, and PAT42) represent regions where markers were uninformative and where phase could not be determined. In the haplotype for the proband, PAT42, a minus sign indicates a deletion determined by loss of a parental allele, and a minus sign within parentheses indicates that a deletion at that locus is inferred on the basis of previous information about both the location of the marker in 17p11.2 and the relation to markers shown to be deleted by FISH studies (see fig. 4).

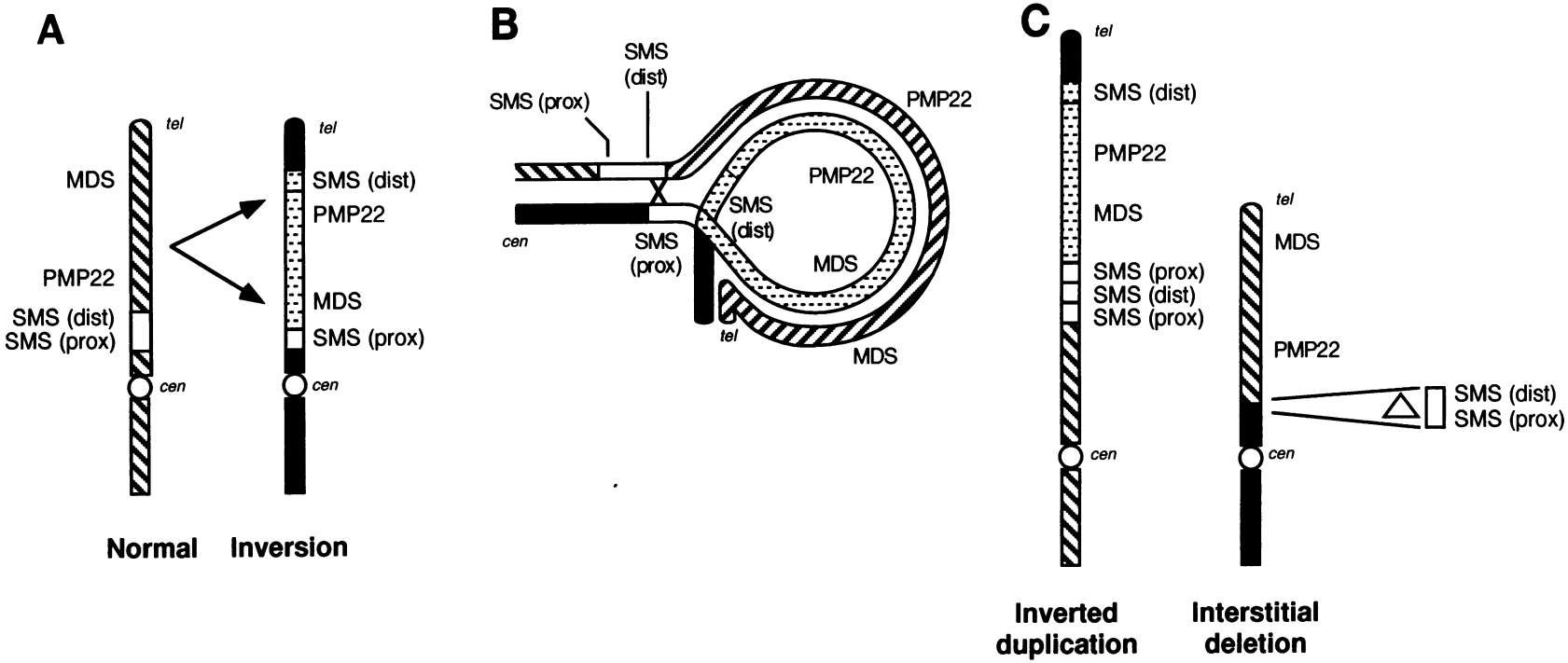

Figure 6 Proposed mechanism of formation of the del(17)(p11.2p11.2) chromosome in paternal meiosis. The symbols "MDS," "*PMP22*," and "SMS" represent the loci for Miller-Dieker syndrome, the peripheral myelin protein 22 gene/Charcot-Marie-Tooth disease type 1A, and the SMS deletion interval, respectively. "(prox)" and "(dist)" refer to the proximal and distal segments of the SMS interval. *A*, Short arms of normal (*hatched portions*) and inversion-bearing (*blackened/dotted portions*) chromosome 17 homologues of the proband's father, PAT44, showing that the inversion event (*dotted portion*) involves only the distal portion of the SMS common deletion region. Note that the SMS common interval not involved in the inversion is denoted by an unblackened box in both homologues, to allow tracking of this region through the recombination event. *B*, Pairing of the homologues shown in *A* such that the inverted region forms a loop, to allow alignment of homologous sequences. An unequal exchange at the bottom of this loop is indicated by a cross (X). C, Products of the unequal exchange—a homologue with an inverted duplication and a homologue that is deleted (Δ) for the entire SMS region. The latter homologue apparently is "reinverted" distal to the deletion, because this portion originates from the grandmaternal noninverted chromosome 17.

explaining the deletion of sequences in the proximal SMS interval in spite of not being involved in the inversion (fig. 6).

A recent review of PAIs concluded that the risk of producing abnormal gametes is expected to be low and directly proportional to the length of the inverted segment (Madan 1995). Since most of the zygotes conceived by these gametes are expected to be nonviable, it is generally believed that PAIs, if adequately distinguished from chromosomal insertions, only rarely result in abnormal viable offspring with recombinant chromosomes (Madan 1995; Sutherland et al. 1995). Our report provides molecular evidence challenging this conventional wisdom and warrants prenatal karyotypic analysis of offspring from a parent with a PAI.

The PAI of 17p seen in this family has been reported only two other times, to our knowledge. In one case, it was ascertained incidentally at amniocentesis and was transmitted by the father (Daniel et al. 1989). That karyotype was 46,XX,inv(17)(p11.2p13.3) and presumably was balanced. The other occurrence was an unbalanced and de novo karyotype 46,XY,inv(17)(p11.1p13) and was associated with the clinical phenotype of SMS (de Rijk–van Andel et al. 1991).

The initial diagnosis of type II SLOS was suspected because of genital and eye abnormalities, postaxial polydactyly of the hands, and syndactyly of toes 2 and 3, combined with early lethality. However, normal cholesterol metabolites and the subsequent identification of the interstitial deletion in chromosome 17p effectively ruled out this possibility. The chromosomal deletion in our patient was typical of SMS, but several of the clinical features were unusual.

Typical SMS patients have a normal life expectancy (Lockwood et al. 1988), with the only known fatality during infancy being reported shortly after cardiac surgery (Smith et al. 1986). The usual phenotype of SMS, at birth, is either normal or characterized by nonspecific features such as hypotonia, congenital heart disease, and Brushfield spots (Finucane et al. 1993). The differential diagnosis includes Down syndrome during infancy, but, as obesity develops during childhood, the phenotype overlaps with Prader-Willi syndrome, especially since strabismus (Finucane et al. 1993) and anomalies of the male genitalia (Stratton et al. 1986; Colley et al. 1990; Moncla et al. 1991) are often seen.

In our patient, the micropenis, cryptorchidism, and abnormal testosterone values after hCG stimulation suggest a defect in either androgen synthesis or global testicular function. Only one female with SMS has been reported to have developed late-onset congenital adrenal hyperplasia (Stratton et al. 1986). Additionally, our patient had an iris coloboma, which is an infrequent but definite finding in SMS (Stratton et al. 1986; Greenberg et al. 1991).

Postaxial polydactyly is considered to be a key diagnostic feature of type II SLOS, but it has also been reported to involve the hands in SMS (Smith et al. 1986; Friedman et al. 1992). Case 3 in the study by Smith et al. (1986) had bilateral ulnar polydactyly, and case 1 in the study by Friedman et al. (1992) had an extra postaxial digit on the left hand. However, clinicians generally do not consider polydactyly to be part of the SMS phenotype, and there was no mention of it in a Japanese report focused on hand findings in SMS (Kondo et al. 1991). Finally, cutaneous syndactyly between toes 2 and 3 is a classic feature of type I and type II SLOS, whereas more generalized syndactyly, involving the hands or the other toes, can occur in SMS (Lockwood et al. 1988; Colley et al. 1990; Allen et al. 1991; Moncla et al. 1991).

In our patient, the combination of polydactyly, micropenis, chordee, cryptorchidism, and death before 2 mo of age seemed atypical for cases of SMS reported to date. This phenotype resembled type II SLOS more than it resembled SMS (except that coloboma was present and hypospadius was absent). Since the clinical features were so atypical for SMS, we were somewhat surprised to find, by molecular studies, that the extent of the deletion was very similar to that seen in typical SMS patients, with involvement of loci flanked by D17S58 and *PMP22* (Juyal et al. 1996). Two SMS patients whose deletions have been characterized as larger than that seen in our patient had milder phenotypes, and their deletions included D17S58 and *PMP22* (Zori et al. 1993; Juyal et al. 1996). One possible explanation for the proband's phenotype might be that a recessive mutation within a gene on the maternal homologue had become unmasked by the deletion on the paternal homologue. Another plausible reason for the difference in phenotype may be that the nature of the sequences involved in the recombination event between the PAI chromosome and the normal homologue may be different from those typically involved in the creation of a de novo interstitial deletion. This may have resulted in distinct position effects or even in disruption of a critical gene that is not typically disrupted in SMS patients.

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