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Tetrasomy 18p de novo: Parental Origin and Different Mechanisms of Formation

Key Words
Tetrasomy 18p · Isochromosome (18p) formation · Parental origin · Nondisjunction · Centromeric misdivision

Abstract
We have used eight PCR-based DNA polymorphisms to determine the parental origin and mechanisms of formation in 9 patients with de novo nonmosaic tetrasomy 18p. The 9 patients, 4 girls and 5 boys, had clinical features characteristic of i(18p) syndrome. The supernumerary marker chromosome was identified by fluorescence in situ hybridization (FISH) analysis using centromeric probes and a flow-sorted 18p-specific library. The isochromosome was of maternal origin in all 9 cases. The formation of tetrasomy 18p cannot be explained by a single model. In 6 cases, meiosis II nondisjunction, followed by subsequent postzygotic misdivsion, and in 1 case postzygotic nondisjunction and postzygotic misdivision were the most likely mechanisms of formation. Alternative mechanisms are suggested in the remaining 2 cases.

Introduction

The first clinical description of a child with a de novo supernumerary isochromosome 18p appeared in 1963 [1]; more than 50 cases have since been published. The tetrasomy 18p syndrome is characterized by psychomotor retardation, microcephaly, long facies with oval shape, high-arched eyebrows, short palpebral fissures and small pinched nose. Facial asymmetry is not unusual. Scoliosis and/or kyphosis, long fingers with contractures and feet malformations are frequent [1–3].

The chromosome 18 specificity of these marker chromosomes can now be confirmed by in situ hybridization with chromosome-18-specific probes [2, 4, 5]. Although previous reports determined only the chromosome 18 derivation of the marker, it can now be unambiguously shown using an 18p-specific library, that the marker chromosome is composed of only short-arm material [6].

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Table 1. Parental ages and clinical findings in 9 cases of de novo tetrasomy 18p

Symptoms	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7	Case 8	Case 9
Birth weight, g	4,000	2,500	3,750	2,700	3,000	3,050	2,940	2,925	2,730
Length, cm	53	48	54	47	56	48	50	48	
Parental age ♀, ♂	25, 31	27, 29	24, 25	37, 41	29, 30	39, 45	28, 23	27, 35	25, 30
Sex	♂	♀	♂	♂	♂	♀	♀	♀	♂
Age at last examination	13 y	20 y	21 y	18 y	4 y	7 y	17 mo	10 mo	8 mo*
Feeding difficulties	+	+	+	+		+	+	+	+
Micro/dolichocephaly	+	+	+	+	+		+		+
Asymmetric face	+		+					+	
Pinched nose	+	+	+			+	+		
Arched eyebrows	+	+	+	+			+		
Narrow palpebral fissures			+	+	+	+/–			
Downslanting eyes	+	+	+	+	+	+			
Epicanthus	+	+			+	+	+	+	+/–
Strabismus	+	+	+			+			+
Dysplastic ears	+	+	+	+	+	+		+	+
High arched palate	+		+	+				+	
Micrognathia			+						+
Slender habitus	+		+	+		+	+		+
Kyphoscoliosis		+	+	+	+				
Clinodactyly/contractures	+	+	+	+	+				
Simian crease	+/–	+/–	+/–		+				–/+
Feet abnormalities	+	+	+	+		+			+
Psychomotor retardation	+	+	+	+	+	+	+	+	+
Epilepsy		+							
Other	cryptor-chism	narrow ear canal	cryptor-chism	hypo-spadias	narrow ear canal	coxa valga	coxa valga		aortic hypoplasia
Earlier published	7 (case 4)		7 (case 2)	8				6 (case 12)	5 and 6 (case 8)

* At autopsy.

Determination of the parental origin of the i(18p)s has previously been hampered by the lack of cytogenetic heteromorphisms of chromosome 18. Several models have been proposed to explain the formation of isochromosomes, but to our knowledge the mechanisms leading to tetrasomy 18p have not been clarified.

We have investigated 9 individuals with de novo tetrasomy 18p and their parents at polymorphic loci to determine the parental origin and mechanism of formation of the additional i(18p) chromosome. The cases were diagnosed by conventional chromosome banding and verified as i(18p)s by fluorescence in situ hybridization (FISH) using a flow-sorted 18p-specific library.

Material and Methods

Patient Population

The 9 patients were 4 girls and 5 boys, referred to cytogenetic analysis because of dysmorphic features and mental retardation. An overview of the patients is given in table 1.

Cytogenetic Studies

Chromosome analysis was performed on cultured lymphocytes using one or more of the following standard methods: QFQ-, CBG-, GTG-, and prometaphase RBA-banding as described [5–8].

Fluorescence in situ Hybridization

Fluorescence in situ hybridization (FISH) analysis was done using a biotin-dUTP-labeled chromosome 18 centromere-specific

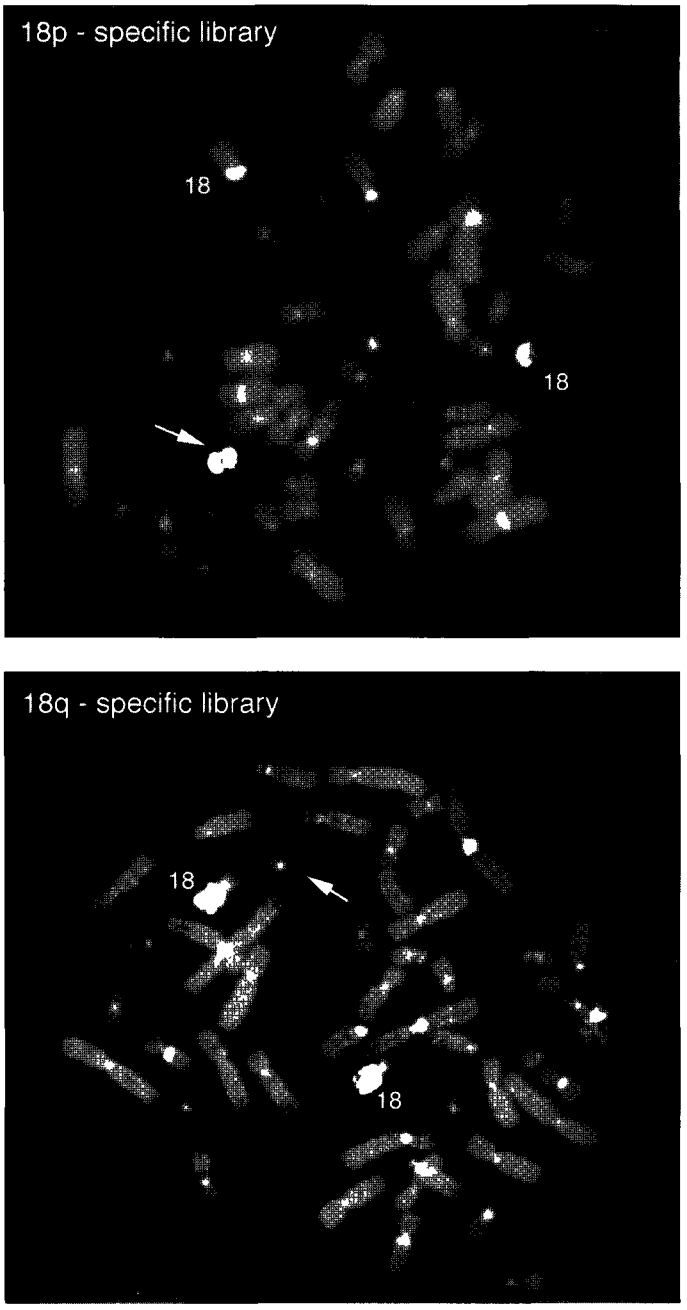


Fig. 1. FISH analysis using an 18p-specific library shows labeling of the p-arms of the two chromosomes 18 and the entire marker chromosome (arrow). Hybridization using an 18q-specific library shows labeling of the q-arms of the two chromosomes 18. The marker chromosome is not labeled (arrow).

probe (ONCOR) according to the manufacturer's instructions. In case 2, in situ hybridization had been performed using a radioactively labeled probe [5].

An 18p-specific library was constructed by flow sorting of an i(18p), and the flow-sorted material was amplified and biotinylated by degenerate oligonucleotide primed-polymerase chain reaction (DOP-PCR) [6]. Probe preparation, hybridization, detection and visualization were performed in all cases by one of us (E.B.) as previously described [6, 9]. An 18q-specific library was also used as control. The construction of this library, labeling, and FISH analysis were performed as above.

DNA Analysis

DNA was extracted from EDTA-anticoagulated blood by a salting-out procedure [10]. Eight DNA polymorphisms were detected after PCR amplification of genomic DNA [11]. End-labeling of primer, PCR amplification conditions, polyacrylamide gel electrophoresis of the amplification product, and autoradiography were performed as described elsewhere [12]. The microsatellites used were $(CA)_n$ repeats at the following loci: D18S170 and D18S40 [13], D18S59, D18S63, D18S54, D18S62, D18S53, and D18S71 [14]. The scoring of polymorphic alleles was performed based on the knowledge of the karyotype showing tetrasomy 18p, i.e. an 18p microsatellite showing homozygosity for one allele in the proband would be interpreted as four copies of the same allele (1111), two alleles of almost equal intensity in the proband would be scored as 1122, two alleles with the smallest allele of much stronger intensity would be scored as 1112, and three alleles with the smallest allele of significantly stronger intensity would be scored as 1123, etc. The scoring was analogous to that of a trisomic condition, where dosage analysis by visual inspection was shown to correlate with densitometric reading of allele intensities [15].

Results

Parental Age Distribution

Range for mothers 24–39 years, mean maternal age 29 years; range for fathers 25–45 years, mean paternal age 32.1 years (table 1).

Chromosome analysis revealed 47 chromosomes in all 9 cases; the size and banding pattern of the extra small metacentric chromosome were compatible with an isochromosome of the short arm of chromosome 18. All cases were nonmosaic. All parental karyotypes were normal.

In situ hybridization with a chromosome 18 centromere-specific probe showed a monocentromeric signal on the marker chromosome in all cases. FISH using an 18p-specific library showed labeling of the short arms of chromosomes 18 and the whole of i(18p). No labeling of the marker chromosome was observed after FISH with an 18q-specific library (fig. 1).

DNA analysis indicated that all nine i(18p) chromosomes were of maternal origin (table 2, fig. 2). An uniparental origin of the normal chromosomes 18 could be excluded. In 6 cases (cases 1–6), the 18p markers showed that the maternal heterozygous alleles were reduced to homozygosity in the proband in the pericentromeric re-

Table 2. DNA polymorphisms on the short arm of chromosome 18 in nine families where the proband has de novo tetrasomy 18p

Locus[1]	Polymorphisms in families[2]								
	case 1			case 2			case 3		
D18S170	14-1223-23	M	NR	13-2233-23		NR	24-1233-13	M	NR
D18S59	11-1233-23	M	NR	23-1133-13		NR	13-1223-12		NR
D18S63	12-1112-11			11-1112-12		NR	11-1223-23	M	NR
D18S54	11-1111-11			12-2344-34	M	NR	12-1223-23		NR
D18S62	34-1123-12	M	NR	12-1122-12		NR	33-1123-12	M	NR
D18S53	23-3333-13		R	23-1112-13	M	R	13-1111-12		R
D18S71	34-2224-12	M	R	13-1444-24	M	R	24-1114-13	M	R
D18S40	23-1113-11	M		11-1222-23	M	R	12-1333-33	M	
CEN	monocentric			monocentric			monocentric		
Error in cell division	nondisjunction maternal meiosis II and postzygotic misdivision								
Locus[1]	case 4			case 5			case 6		
D18S170	23-1124-14	M	NR	23-1124-14	M	NR	34-1124-12	M	NR
D18S59	11-1112-12		NR	34-1124-12	M	NR	13-2333-23		NR
D18S63	11-1111-11			12-1113-13		NR	12-1223-23		NR
D18S54	23-1223-12		NR	13-1223-12		NR	13-1224-24	M	NR
D18S62	13-1233-23		NR	12-1111-11			22-1123-13	M	NR
D18S53	11-1222-23	M	R	34-2223-12	M	R	11-1111-12		R
D18S71	12-1111-13		R	12-1333-13	M	R	12-1222-23		R
D18S40	12-1111-11			11-1111-12		R	14-1222-23	M	R
CEN	monocentric			monocentric			monocentric		
Error in cell division	nondisjunction maternal meiosis II and postzygotic misdivision								
Locus[1]	case 7			case 8			case 9		
D18S170	12-1223-23		NR	23-1112-11	M		34-1113-12	M	R
D18S59	14-1223-23	M	NR	23-1123-13		NR	13-2223-12	M	R
D18S63	12-1112-12			12-1334-34	M	NR	12-1111-12		R
D18S54	12-2233-23		NR	13-2233-23		NR	13-1113-12		R
D18S62	12-1111-11			13-1123-12		NR	12-1222-22		
D18S53	12-2333-33	M		33-1223-12	M	NR	13-3444-24	M	R
D18S71	13-1223-12		NR	12-1111-11			12-1111-11		
D18S40	12-2222-22			12-1112-11			12-1333-34	M	R
CEN	monocentric			monocentric			monocentric		
Error in cell division	NDJ MI and misdivision MII or premeoitic misdivision and NDJ MI						postzygotic NDJ and misdivision		

NDJ = Nondisjunction; MI = maternal meiosis I; MII = maternal meiosis II.

[1] The loci on the short arm of chromosome 18 are ordered in a column corresponding to their relative order based on linkage analysis [16].

[2] The three parts of the data correspond to father, proband, and mother, respectively. The numbers in the genotypes represent the different alleles at a specific locus. M = Maternal origin of the additional i(18p) as determined by the respective DNA polymorphisms. R, NR = reduction to homozygosity and nonreduction to homozygosity, respectively, for a given locus.

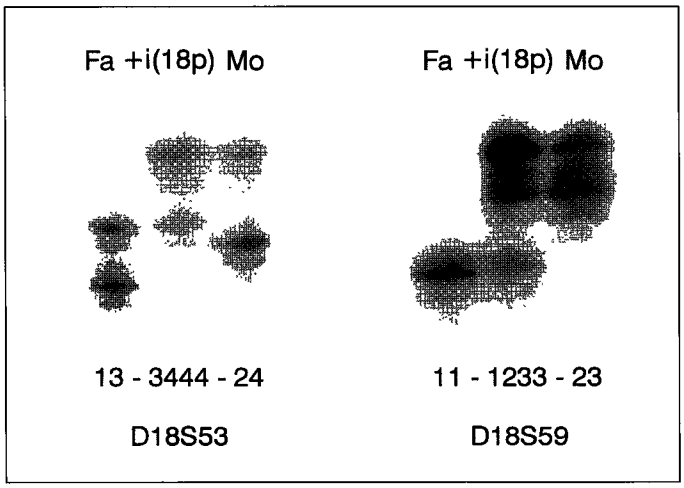


Fig. 2. Determination of parental origin of additional chromosome i(18p) with the dinucleotide repeat D18S53 and D18S59 in families where the proband has tetrasomy 18p. The genotypic scoring of alleles is indicated in each individual. Fa = father; +i(18p) = tetrasomy 18p proband; Mo = mother.

Discussion

In their original report, published in 1963, before the banding era, Frøland et al. [1] suggested that the extra chromosome was an isochromosome of the short arms of chromosome 17 or 18. Later, with banding an isochromosome for 18p was suspected. However, by classical chromosome analysis it is sometimes difficult to distinguish an i(18p) from a deleted chromosome 18 [4, 6–8].

In the present cases, FISH analysis with a centromere-specific probe as well as with 18p- and 18q-specific libraries, showed that the marker chromosome was monocentric, and that it was unequivocally an i(18p), since the whole of the marker was painted with the 18p-specific library, with no labeling using the 18q library (fig. 1).

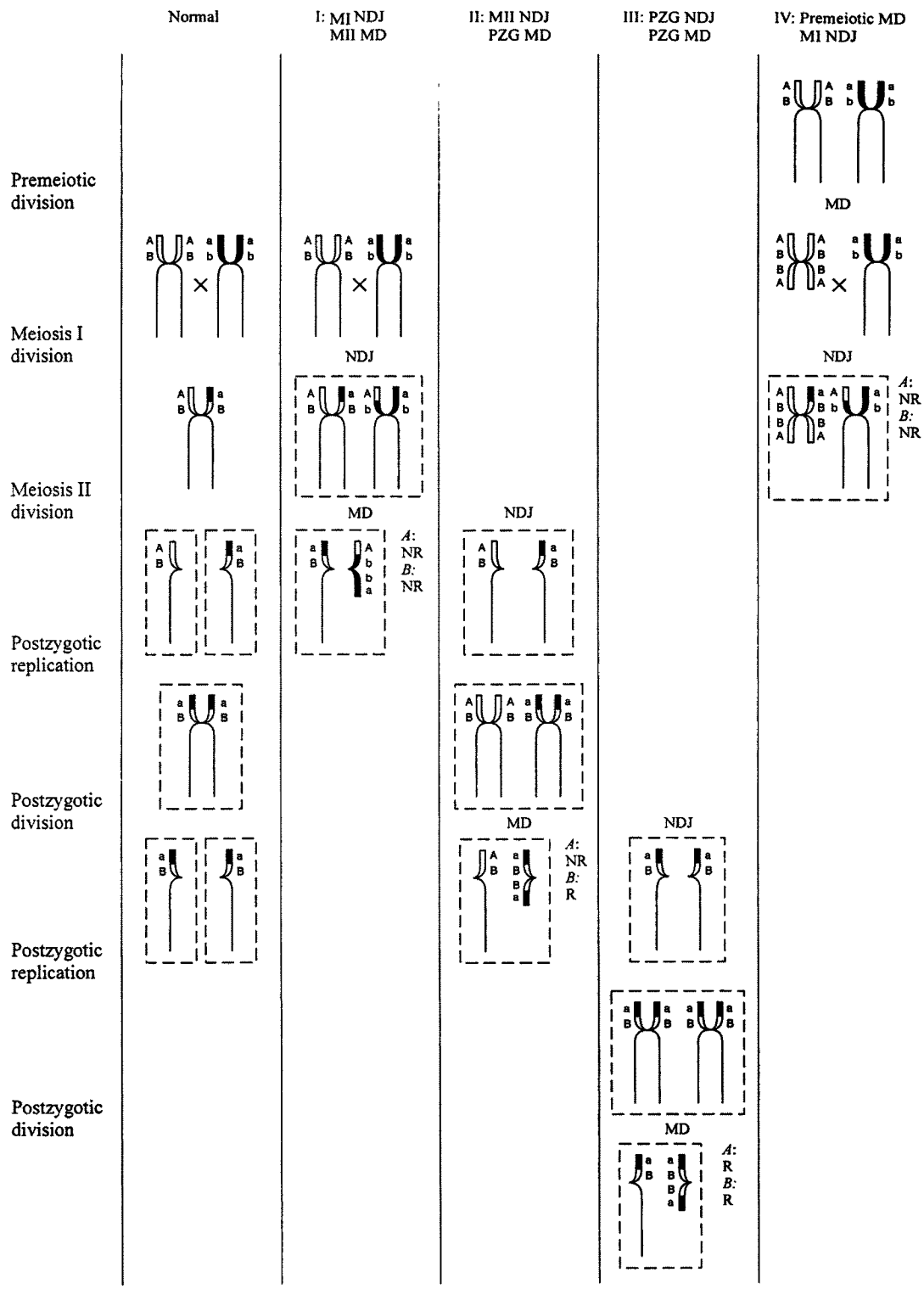
Formation of a nonmosaic de novo tetrasomy 18p due to an additional monocentric isochromosome 18p requires two events: nondisjunction and centromeric misdivision (= mitotic centromeric fission with fusion of sister p-arms).

The segregation of polymorphic DNA markers in different categories of premeiotic, meiotic or mitotic errors is presented in figure 3. The DNA markers used in this study cover the entire length of the short arm of chromosome 18 [16]. In 6 cases (cases 1–6), we found that the maternal heterozygous alleles were reduced to homozygosity in the pericentromeric region in the proband but retained heterozygosity in the telomeric region (table 2). To explain those 6 cases, we propose a model in which the tetrasomy is a result of meiosis II nondisjunction followed by postzygotic misdivision of one of the chromosomes with subsequent loss of all long-arm material (fig. 3, II).

An alternative series of events compatible with the data is a premeiotic (mitotic) nondisjunction followed by a centromeric misdivision in MII [17].

In case 9, 6 of the 8 DNA markers were informative and showed reduction to homozygosity of the maternal alleles in the proband (table 2). Only postzygotic nondisjunction of chromosome 18 followed by misdivision of one of the chromosomes can explain these results (fig. 3, III). This assumes that no marker between the centromere and D18S40 shows nonreduction to homozygosity of maternal alleles. A postzygotic misdivision of one of the chromosomes in a maternally derived trisomy 18 may theoretically occur on the paternal chromosome in one third of the cases. Such cases would have maternal uniparental disomy for the long arm of chromosome 18. We tested this possibility with DNA markers mapping to the long arm of chromosome 18. At three informative 18q loci: D18S36, D18S35, and D18S38 biparental inheritance was demonstrated (data not shown). Usually mosaicism would be expected in cases of postzygotic nondisjunction. Analysis of 20 mitoses in blood and 59 mitoses in fibroblast cultures did not disclose any mitosis lacking the i(18p).

Fig. 3. Schematic representation of the segregation of a locus *A* near the telomere and a locus *B* near the centromere, each with two alleles (A, a and B, b) in normal meiosis and following premeiotic, meiotic, or postzygotic errors. Cross-over in meiosis I is indicated with ×. Not all the products of meiosis are shown. R = Reduction to homozygosity in the individual with tetrasomy 18p, given that the polymorphism was heterozygous in the parent who contributed the supernumerary isochromosome 18p; NR = nonreduction to homozygosity in the individual with tetrasomy 18p, given that the polymorphism was heterozygous in the parent who contributed the supernumerary isochromosome 18p; MI = meiosis I; MII = meiosis II; PZG = postzygotic division; NDJ = nondisjunction; MD = centromeric misdivision.


Normal
I: MI NDJ
MII MD
II: MII NDJ
PZG MD
III: PZG NDJ
PZG MD
IV: Premeiotic MD
MI NDJ
Premeiotic division
Meiosis I division
Meiosis II division
Postzygotic replication
Postzygotic division
Postzygotic replication
Postzygotic division
NDJ
MD
A: NR
B: NR
A: NR
B: R
A: R
B: R

3

In 2 cases (cases 7 and 8), the probands were heterozygous for all informative maternal alleles. Unfortunately, the one or two markers, respectively, located closest to the centromere were not informative as the mothers were homozygous (table 2). If the maternal alleles in these probands were all nonreduced, the i(18p) formation could be explained by two previously postulated mechanisms: a centromeric misdivision occurring during a germinal premeiotic mitosis followed by MI nondisjunction [18] (fig. 3, IV), or an MI nondisjunction followed by a centrometric misdivision of one of the univalents during meiosis II [19] (fig. 3, I).

The proposed models for formation of the tetrasomy 18p are based on deduced haplotypes for the normal chromosomes 18 and the isochromosome 18p. However, in cases 7 and 8 alternative haplotypes could be determined from the pattern of the polymorphisms in the families. Determination of the i(18p) haplotypes, e.g. by PCR analysis of flow-sorted or microdissected i(18p) chromosomes would allow us to verify whether the deductions are correct or not.

Nondisjunction of chromosome 18 must be involved in the formation of tetrasomy 18p. DNA studies of trisomy 18 have shown that the majority of cases result from a maternal MII error [16]. This is in agreement with the most common model we propose, invoking MII nondisjunction as the first step in the formation of tetrasomy 18p.

Molecular analyses have also demonstrated that paternally derived trisomy 18 occurs in less than 5% of cases [16, 20]. In the present study, the isochromosome 18p was of maternal origin in all 9 cases.

Trisomy 18 is associated with advanced maternal age. Fisher et al. [16] found that the maternal age effect is significant in the MII error group. So far, an association of advanced parental age with tetrasomy 18p has not been demonstrated. In 1978, Nielsen et al. [7] found a mean maternal age of 30 years for the 25 cases of tetrasomy 18p reported previously. However, none of these papers explained the mechanisms involved in the i(18p) formation. Since we invoke that maternal MII nondisjunction is involved in several of the cases, increased maternal age would be expected. However, in the present limited number of cases, maternal age was increased in only 2 cases (cases 4 and 6, table 1).

The results of the present study serve to demonstrate that no single mechanism can account for all i(18p) cases. Based on genotyping, both premeiotic, meiotic and postzygotic division errors can be involved alone or in combination. Further studies are needed to determine the parental origin and to clarify the mechanisms of formation.

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Note Added in Proof

In this issue of the journal, Kotzot et al., pp. 168–174, have published the results of their examination of 9 probands with additional 18p. In most of the cases, if not all, the marker chromosome originated from maternal meiosis II nondisjunction followed by isochromosome formation in one of the two maternal chromosomes 18, as observed in the majority of cases in our study. Our results indicate that not all cases are a result of this mechanism since 3 of our probands show different mechanisms of formation. In contrast to Kotzot et al., we saw increased maternal age only in 2 of the 9 cases.