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Understanding the Mechanism(s) of Mosaic Trisomy 21 by Using DNA Polymorphism Analysis

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Summary

In order to investigate the mechanism(s) underlying mosaicism for trisomy 21, we genotyped 17 families with mosaic trisomy 21 probands, using 28 PCR-detectable DNA polymorphic markers that map in the pericentromeric region and long arm of chromosome 21. The percentage of cells with trisomy 21 in the probands' blood lymphocytes was 6%–94%. There were two classes of autoradiographic results: In class I, a "third allele" of lower intensity was detected in the proband's DNA for at least two chromosome 21 markers. The interpretation of this result was that the proband had inherited three chromosomes 21 after meiotic nondisjunction (NDJ) (trisomy 21 zygote) and subsequently lost one because of mitotic (somatic) error, the lost chromosome 21 being that with the lowest-intensity polymorphic allele. The parental origin and the meiotic stage of NDJ could also be determined. In class II, a "third allele" was never detected. In these cases, the mosaicism probably occurred either by a postzygotic, mitotic error in a normal zygote that followed a normal meiosis (class IIA mechanism); by premeiotic, mitotic NDJ yielding an aneusomic zygote after meiosis, and subsequent mitotic loss (class IIB mechanism); or by a meiosis II error with lack of crossover in the preceding meiosis I, followed by mitotic loss after fertilization (class IIC mechanism). Among class II mechanisms, the most likely is mechanism IIA, while IIC is the least likely. There were 10 cases of class I and 7 cases of class II results. Within class I, there were nine cases with maternal meiotic errors (six meiosis I and three meiosis II errors, on the basis of pericentromeric markers) and one with paternal meiosis I error. The postzygotic loss of chromosome 21 was determined in eight maternal class I cases, and it was maternally derived in five cases and paternally derived in three; this suggests that the postzygotic loss of chromosome 21 is probably random. The mean maternal age in meiotic class I errors was 31.4 years and in mitotic class II errors was 27.4 years, as expected.

Introduction

Trisomy 21, the most common viable chromosomal abnormality in humans, with a frequency of ~1:650 live births, is the cause of Down syndrome (DS) (Lejeune et al. 1959; Hook 1982), which includes mental retardation, characteristic physical features, and reduced life expectancy. About 90%–95% of DS cases are due to free trisomy 21, which has long been known to be associated with advanced maternal age. Another 2%–4% have chromosomal rearrangements such as translocations with acrocentric chromosomes resulting in trisomy 21 (for review, see Epstein 1989). The remaining 2%–4% of DS patients show mosaicism for trisomy 21 and normal cell lines (Mikkelsen 1977). In this group there is also a maternal age effect, although to a lesser degree than in nonmosaic free trisomy 21 (Richards 1969). Detection and determination of the frequency of the aberrant cell line in mosaics depends on the number of cells and tissues examined and on in vivo selec-

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Table 1

Analysis of DNA Polymorphisms in Families with Mosaic Trisomy 21

	DSmos1	DSmos2	DSmos3	DSmos4	DSmos5	DSmos6	DSmos8	DSmos9	DSmos10
Mosaicism (%)	6	7	8	8	9	10	12	17	26
Maternal age/paternal age (years)[c]	29/29	28/33	38/29	38/39	22/25	28/27	21/32	28/29	24/30
Origin of trisomy 21[d]	M1	Mitosis	Mitosis	P1	Mitosis	M1	Mitosis	Mitosis	Mitosis
Lost allele[e]	m			p		p			
Crossover event detected?	No			No		Yes			
Chromosome 21 markers:									
D21S215	23.12.23(1) m	13.22.12	12.11.11	12.00.11	12.11.11	24.13.13(4) Mp	00.12.12	11.22.12	22.12.22
D21S258	12.11.11	23.12.13	23.13.13[f]	11.23.13	34.12.14	11.00.11	22.13.12	12.22.13[f]	12.23.22
D21S236	12.22.12	11.11.11	23.12.22	22.13.23	12.11.12	22.00.13[f]	00.11.11	11.23.12	12.33.13
D21S120	22.12.22(1) m	12.12.12	11.22.12	22.11.12	13.23.13 x	11.00.12	13.12.12	12.22.12	22.12.12
D21S13		11.11.11	11.22.12	23.12.12(3) p	12.22.12	12.00.12	00.13.23 x	12.22.12	
D21S172	11.12.12	12.12.11	23.14.34[f]	13.23.33(1) p	34.12.14[f]	12.12.12	12.34.24[f]	22.11.12	14.23.34
D21S11	12.34.24(3) Mm	14.23.13	22.11.12	23.12.23	13.12.23	13.00.23	12.23.22	23.13.13[f]	
D21S145									
D21S214	11.11.11	23.14.12	13.12.13	23.12.23	23.12.23	13.24.24(1) Mp	22.12.22	23.11.12	23.11.13[f]
D21S1244	12.22.00	00.12.12	12.11.11		33.12.13	13.22.22(3) Mp	13.12.13	12.33.23	13.22.23
D21S222	11.11.11	23.13.33	11.11.11	23.13.23	13.12.11 x	13.24.24(1) p	33.12.23[f]	11.12.12	23.12.12
D21S232	11.22.12	12.22.22	12.23.12	12.00.12		23.13.13	12.22.12	11.22.12	13.23.12
D21S210	00.12.12	12.11.12	12.34.24[f]	12.00.11(2)	12.23.22		22.13.23[f]	23.12.22[f]	23.13.23
APP			14.23.12			22.00.12	22.11.12		
D21S217	24.13.23[f]	12.13.13	11.22.12	12.34.24(1) Pp	12.22.22	23.13.13	12.22.22	13.12.13[f]	33.12.13[f]
D21S1239	11.11.11	11.12.11	11.11.11	12.11.12	13.23.23[f]	12.11.11	11.12.11	12.11.11	12.12.12
D21S213					22.11.12		00.12.12	22.11.12	
D21S216	22.12.22[f]	22.12.12	12.33.23[f]	11.12.12	12.12.12	13.22.22[f]	22.12.22[f]	12.11.12	12.12.12
IFNAR	11.23.12[f]	23.12.23	22.11.12	22.12.12	24.13.14	12.13.13(2) p	23.13.23		23.13.13
GART		12.33.13	23.12.12			11.22.22(1) Mp		11.00.12	13.24.14 x
D21S65	22.13.12[f]	11.11.11	22.11.12	22.11.12	23.13.13 x	11.12.12	12.11.11	23.12.12	14.23.12
D21S167	00.12.12	12.22.12	23.13.33[f]	13.12.11(3) p	12.13.13	12.12.12		14.23.12	12.11.11
D21S156	12.13.13[f]	24.13.14	12.11.12	13.23.33(1) p	11.12.11	13.24.24(3) Mp	23.14.12 x	34.22.24[f]	23.12.22
HMG14	12.13.13	23.11.13	11.12.12	12.13.11(2) p	23.14.24 x	13.12.12	23.11.12	12.12.12	12.13.11
D21S231	23.11.13[f]	13.24.34	11.23.13	13.12.11(3) p	12.34.14	12.11.11	12.12.11	11.12.12	12.12.12
D21S212	12.12.22(1)	12.12.11	13.12.11		12.23.23	11.12.12	12.23.23	12.23.12	14.23.12
D21S170	11.12.12	11.12.12		12.13.11(2) p	12.13.12	14.23.22(4) Mp	23.11.12	11.12.12	12.23.23[f]
PFKL	23.14.34[f]	11.12.12	12.22.22[f]	11.11.11	23.11.12 x	11.12.22	00.11.12	12.12.12	22.13.12
Non-chromosome 21 markers:									
D8S262			12.22.22	12.34.14		13.22.12			
D8S264	12.34.24								13.12.12
D8S89	23.12.12						11.11.11		
UT975 (chromosome 5)	12.23.12	23.13.13		12.00.12	22.13.12	11.11.11	34.12.14	11.12.11	11.23.13
UT658 (chromosome 10)	12.23.22	24.13.12		14.23.34	34.12.13	14.23.13		13.24.12	12.11.12

NOTE.—The chromosome 21 polymorphic markers are listed as they appear on the linkage map, from the most centromeric D21S215 to the most telomeric PFKL. Markers proximal to D21S13 are considered representative of the centromere, for the determination of the meiotic stage of NDJ. Allele number in parentheses indicates the polymorphic allele with the lowest intensity in the autoradiogram and marks the lost chromosome 21. The genotypes from the DNA of the father, mother, and trisomy 21 proband are presented. For example, in family DSmos1, the genotypes for marker D21S215 were as follows: father's DNA showed alleles 2 and 3, mother's DNA showed alleles 1 and 2, and proband's DNA showed alleles 2, 3, and 1 with reduced intensity. x = exposure of the film was not sufficient to exclude the existence of a "third allele"; R = reduction to homozygosity for alleles from the parent that contributed two chromosomes 21; for other abbreviations, see notes d and e, below.

[a] Determined from an amniotic fluid sample.

[b] Determined from a sample taken in 1976; the sample used for DNA extraction was obtained in 1990, and its percentage of mosaicism is unknown.

[c] At birth of the mosaic trisomy proband.

[d] M = maternal origin; P = paternal origin; M1 = error in maternal meiosis I; M2 = error in maternal meiosis II; P1 = error in paternal meiosis I; and "mitosis" = supernumerary chromosome 21 was the result of a mitotic error (mechanism II; see Discussion).

[e] m = loss of maternal chromosome; and p = loss of paternal chromosome.

[f] Possibility of no recognition of the "third allele," because of "stuttering" of the polymorphic bands.

[g] Only this marker was informative for intensity difference among the three alleles.

DSmos11	DSmos12	DSmos13	DSmos14	DSmos15	DSmos16	DSmos17	DSmos18
13	15	88	10	11	50	94[a]	≈10[b]
31/35	43/52	32/34	29/33	28/28	28/31	35/33	31/32
Mitosis	M2	M1	M2	M1	M2	M1	M1
	p	p	m	m	m	?	m
	Yes	No	Yes	No	Yes	No	No
12.22.12	12.22.22	12.12.122	12.12.00			23.12.122	11.12.112
23.11.12	22.13.33[f]	12.00.23(1)	12.34.13 R	12.23.13[f]	23.13.13	23.13.133	12.11.111
12.34.13	12.11.11[f]	11.11.11	11.23.12 R	13.23.33[f]	23.13.33 R		22.12.122
22.12.22	12.12.11 R	11.11.11	23.12.13 R	11.11.11	13.22.12	11.12.112	22.12.122
23.00.12	11.12.22(1) MR	13.12.12(3) p	12.12.12	23.13.33(1)	33.12.13[f]		11.12.112
12.22.22	12.23.22 R	11.11.11	12.12.00	23.13.13	11.23.13	23.12.122	23.12.122
34.12.14[f]	13.12.11 R		23.12.12[f]	14.23.12(3) Mm	22.12.12	12.12.112	14.23.12(3) Mm
						11.23.23(1)[g]	11.23.12(3) Mm
11.23.13	12.22.00	24.13.13(4) Mp	13.24.34 R	13.23.23[f]	13.12.12 x	13.22.223	11.23.13(2) Mm
13.22.23	12.12.12	13.00.12(3)	11.22.12	13.24.34(2) Mm	22.12.12		
23.14.13	23.12.12	23.13.123	11.11.11	24.13.12(3) Mm	14.23.24[f]	11.12.112	12.13.23(1)
12.11.12	11.12.12	12.00.11	12.12.12	11.12.12	11.22.12	12.22.122	11.12.112
12.34.13[f]	13.22.22[f]	11.12.112	12.12.12	12.33.23[f]	23.13.23[f]		
23.13.33	13.22.22[f]	13.12.112	11.23.12 R	12.34.24(3) Mm	23.14.24[f]		
11.11.11	12.12.12	11.00.11	11.11.11			13.12.123	12.13.23(1) Mm
			00.12.00	22.12.12	12.23.22(3) m		
12.12.12	11.12.12	23.12.122	11.12.12	11.11.11	12.12.22		
12.33.13	23.12.12	12.00.112	12.22.12	23.11.13	12.23.13(2)		
22.12.22	23.13.13[f]	22.13.13(2) Mp	12.13.13	13.12.23[f]	23.12.22		
24.13.12	34.12.12(3) Mp	13.24.234 M	11.23.13(2) Mm	12.23.13 x	24.13.34 e	13.23.123	23.14.13(4) Mm
11.22.12	12.13.13(2) Mp	13.00.23(1)	12.11.11	34.12.23 x	14.23.24[f]	13.24.234 M	13.12.112
23.14.12	12.34.34(2) Mp	11.12.12	34.12.23[f]	12.34.24[f]	13.12.13 e	14.23.123 M	
12.13.13[f]	13.22.22(1) Mp	24.13.13(2) Mp	23.14.12(4) Mm	23.11.12	13.12.23(1)	23.12.122	
11.12.12	11.12.22(1) M	23.12.12(3)	11.11.11	11.11.11	11.23.12(3) Mm		
33.12.23[f]			12.33.13[f]	12.33.13	12.13.23(1)	13.22.223 M	
23.12.13[f]	12.11.11	13.24.24(1) Mp	33.12.23[f]	12.22.12	13.12.23[f]	12.11.111	12.23.223
12.11.11	12.22.22	12.12.122	12.23.13[f]	12.23.12(3) m	13.22.23 x	13.24.124 M	11.23.13(2) Mm
11.11.11	11.12.12		12.22.22	13.12.13	12.22.22		
22.13.23			11.22.12	12.33.13	12.13.11		
		12.12.12	12.23.22	23.12.13	23.13.23		
11.12.11	12.13.13	12.33.23	11.23.13	12.23.22	11.23.12		
12.11.12	13.24.12	13.00.12					

tion of one of the cell lines (Taylor 1968, 1970). Speculations have been made in the past on the mechanisms responsible for the production of mosaic trisomy 21. On the basis mainly of observations of maternal age, Richards (1969, 1974) predicted that ~80% of the errors leading to mosaic trisomy 21 may be due to loss of the supernumerary chromosome of a trisomic zygote, while the remaining 20% probably result from mitotic nondisjunction (NDJ) of an euploid zygote. He predicted that, in the first class, the mean maternal age should be comparable to that for free trisomy 21, while in the second class, it should not be different from the mean maternal age for the general population. Chromosomal heteromorphisms of the short arm of chromosome 21 have been used to investigate the parental and meiotic origin of the additional chromosome in the trisomic cell lines of a few patients with mosaic trisomy 21 (Magenis and Chamberlin 1981; Niikawa and Kajii 1984). A total of five cases were studied, three of which seemed to originate from a first meiotic NDJ event, while two probably resulted from mitotic events. In one study of the origin of the extra chromosome 21 in 343 families with DS, wherein both chromosomal heteromorphisms and DNA polymorphisms were used, 24 cases of mosaic trisomy 21 were included (Dagna-Bricarelli et al. 1990). No precise description was given of the methods used in the mosaic cases or of the specific results obtained in these families.

The goal of our study was to estimate the frequency of the two main mechanisms that produce mosaic trisomy 21: (I) mitotic loss of the supernumerary chromosome 21 from a trisomic zygote resulting from a meiotic NDJ and (II) mitotic duplication of a chromosome 21, occurring either post- or premeiosis. To investigate the mechanisms related to mosaicism for trisomy 21, we analyzed PCR-detectable short-sequence repeat (SSR) polymorphisms (Weber and May 1989) that map on the entire long arm of human chromosome 21, from the most centromeric D21S215 to the most telomeric PFKL (McInnis et al. 1993). The power of PCR in detecting low-copy-number sequences and the abundance of markers genotyped permitted the classification of all cases studied.

Subjects and Methods

Patients and Their Parents

Our sample consisted of 18 families with mosaic trisomy 21 probands. For each family, blood samples from the father, mother, and affected child were obtained; the same blood sample was used for extraction of DNA and for cytogenetic analysis, except for family DSmos18. In case DSmos17, the sample was from cultured amniotic fluid. The percentage of cells with trisomy 21, as derived from cytogenetic analysis of blood leukocytes, was 6%–94% (see table 1).

DNA Polymorphism Analysis

The DNA polymorphisms used in the present study and mapped on chromosome 21 were D21S215, D21S258, D21S236, D21S120, D21S13, D21S172, D21S11, D21S145, D21S214, D21S1244 (UT761), D21S222, D21S232, D21S210, APP, D21S217, D21S1239 (UT489), D21S213, D21S216, IFNAR, GART, D21S65, D21S167, D21S156, D21S231, HMG14, D21S212, D21S170, and PFKL (for information concerning the oligonucleotides used per marker and their mapping positions, see the Genome DataBase; Avramopoulos et al. 1993; McInnis et al. 1993). Genotypes for all markers were obtained by using PCR amplification and PAGE, as described elsewhere (Petersen et al. 1991; Antonarakis et al. 1992). In brief, 100–200 ng of DNA were used as template for PCR amplification (Saiki et al. 1985), and the conditions for the reaction were 94°C for 6 min and then 25–29 cycles of 94°C for 30 s, 50°C for 30 s, and 72°C for 30 s, for all the markers used. In some cases, 30–35 cycles of PCR amplification were used for better visualization of the alleles with low copy numbers (no artifacts interfering with the allelic bands were noted after PCR amplification with >30 cycles). The forward primer was end-labeled with γ-^{32}P ATP by using T4 kinase, and the PCR product was electrophoresed in 6% polyacrylamide/6 M urea in 1 × Tris-borate–EDTA for 2–3 h. The dried gel was autoradiographed for 24 and 72 h (two exposures). The interpretation of the data from the genotypes is explained in Results.

To verify that the "third allele" polymorphic band of chromosome 21 markers was not the result of sample contamination with exogenous DNA, we determined the genotypes of five non–chromosome 21 polymorphic markers, namely, D8S262, D8S264, D8S89 of chromosome 8 (see Genome DataBase for oligonucleotides and mapping information), UT975 of chromosome 5, and UT658 of chromosome 10 (unpublished markers from S. Gerken and R. White; Avramopoulos et al. 1993). The genotypes of non–chromosome 21 polymorphic markers were also used to verify paternity (see Discussion).

Cytogenetic Analysis

Chromosome analysis was performed from 72-h cultures of blood leukocytes. Chromosomes were banded

by the RHG or GTG technique. At least 100 mitoses were analyzed for each individual, including patients and parents. When only 1 cell was found to be trisomic, a total of 200 metaphase cells were examined. We considered mosaicism to be the existence of at least 2 trisomic cells in the 200 metaphase cells examined. No cytogenetic heteromorphisms were studied, because of their disadvantages when compared with SSR polymorphisms (Antonarakis et al. 1991; Petersen et al. 1992).

Results

A total of 18 cases were examined. The percentage of mosaicism in the patients, as derived from cytogenetic analyses, varied from 6% in case DSmos1 to 94% in case DSmos17 (table 1). All but one of the parents were found to have normal karyotypes without mosaicism for trisomy 21 cells. The father of family DSmos11 was found to have three different cell lines: 46,XY/47,XY+21/45,XY-21 (98.25%/0.75%/1%, respectively, in 400 metaphases examined).

A total of 28 markers spanning the entire long arm of human chromosome 21 were analyzed, and the results are shown in table 1. In one family, DSmos7, nonpaternity was detected with both chromosome 21 and non–chromosome 21 markers; this family was eliminated from further analysis. There were two main classes of results from the DNA polymorphism analysis, the interpretation of which follows.

Class I cases are those in which three different alleles are present in the DNA of the individual with mosaic trisomy 21. In these instances, the allele that showed least intensity in the autoradiogram (see fig. 1 for examples) is considered to mark the chromosome that was lost from the trisomic cell line that became disomic. We therefore interpret this case as a meiotic error (since there were three different alleles in the trisomic cell line) and the mosaicism as due to mitotic (somatic) loss of one of the three chromosomes 21. The lost "third allele" is shown in parentheses in table 1. In these cases, the error was assigned to meiosis I or meiosis II according to the data obtained by using a considerable number of pericentromeric DNA polymorphisms, as described elsewhere (Antonarakis et al. 1991, 1992; Petersen et al. 1991; Pangalos et al. 1992).

Class II cases are those in which a third distinctive allele for chromosome 21 markers was never visualized in the DNA of the proband. In the majority of such cases, the rate of mosaicism for the trisomy 21 cell line was ~10%; this percentage did not permit clear allelic dosage differences to be seen in autoradiograms. The

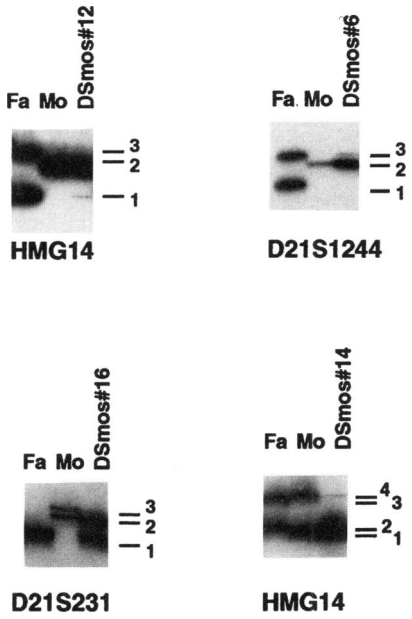

Figure 1 Examples of autoradiograms from the DNA polymorphism analysis of families with mosaic trisomy 21. The first example shows the results of marker HMG14 in family DSmos12. "Fa" denotes DNA from the father, and "Mo" denotes DNA from the mother. The DNA from the proband is designated "DSmos12." The scoring of this polymorphism is 13.22.22 (1), i.e., alleles 1 and 3 in DNA from the father, homozygosity for allele 2 in DNA from the mother, and alleles 2 and (1) in DNA from the proband. Allele 1 in the proband shows very low intensity in the autoradiogram and is considered to be the allele present on chromosome 21 that was lost from the trisomic cell line. Such alleles are shown in parentheses in table 1. In the second example, marker D21S1244 in family DSmos6, the "lost allele" is allele 3. The "lost alleles" in the other two examples are as follows: D21S231 (DSmos16) allele 3 and HMG14 (DSmos14) allele 4.

exceptions were in cases DSmos13 and DSmos17, where the high percentage of mosaicism (88% and 94%, respectively) permitted the visualization of "three copies" of chromosome 21 markers when only two allelic polymorphic bands were present on the autoradiogram, and in case DSmos18, where the percentage of mosaicism in the sample from which the DNA polymorphism data were obtained was unknown. In cases where a third distinct allele was never detected, the interpretation of the data was that there was a normal disomic zygote in which chromosome 21 was subsequently duplicated during mitotic divisions, thus creating a cell line with trisomy 21 (mechanism IIA), or a premeiotic mitotic error in the parental germ cells and subsequent mitotic postzygotic loss (mechanism IIB), or a meiosis II error with lack of crossover in the preceding meiosis I,

Table 2

Mechanism of Mosaicism for Trisomy 21: Summary of Results

Mechanism	No.	Mean Maternal Age (years)	Lost Chromosome 21		
			m	p	Unknown
Meiotic T21 (class I mechanism)	10				
Maternal origin	9	31.4	5	3	1
M1	6		3	2	1
M2	3		2	1	
Paternal origin	1				
P1	1			1	
P2	0				
Mitotic T21[a] (class II mechanisms)	7	27.4			

NOTE.—For abbreviations m, p, M1, M2, and P1, see table 1; P2 = error in paternal meiosis II.
[a] The class IIC mechanism is meiosis II and mitosis (see text).

followed by a mitotic loss after fertilization (mechanism IIC).

Table 2 contains the results of the study based on the interpretations described above. There were 10 cases (58.8%) in which the mosaicism probably originated from a postzygotic loss of an extra chromosome 21 present in a trisomic zygote (class I mechanism). In the remaining seven cases (41.2%), the mosaicism seemed to be the result of a mitotic, postzygotic gain of an extra chromosome 21 in a previously euploid zygote (class IIA mechanism); or of a premeiotic duplication in the parental germ cell line, leading to a trisomic zygote and subsequent loss of the supernumerary chromosome 21 (class IIB mechanism); or of a meiosis II error without a crossover in meiosis I and with a mitotic loss after fertilization (class IIC mechanism). The family in which the father was also found to be mosaic for trisomy and monosomy 21 (DSmos11) probably belongs to class IIB. In the class I cases, the supernumerary chromosome 21 was of maternal origin in nine cases and of paternal origin in one. Of the nine maternal cases, six were attributed to meiosis I errors and three to meiosis II errors (the inference of meiosis I or meiosis II errors was based on informative pericentromeric DNA polymorphisms from D21S215 to D21S13). In the paternally derived trisomy 21, the error occurred in meiosis I. The mean maternal age at birth for the meiotic errors of class I was 31.4 (SD 4.9) years, and for class II it was 27.4 (SD 5.9) years. The small number of individuals does not allow statistical confidence (Student t-test; $P = .159$), but the results agree with what should be expected. In the 10 cases where the zygote was trisomic, the polymorphic marker study permitted the determination of the parental origin of the lost chromosome 21 in nine cases. The data indicated that the postzygotic (mitotic) loss of a chromosome 21 involved a paternal chromosome in four cases and a maternal chromosome in five. Of the four cases where the lost chromosome was paternal, the error that produced the trisomy was in maternal meiosis I in two cases, in maternal meiosis II in one, and in paternal meiosis I in one. Of the five cases where the lost chromosome was maternal, the error that produced the trisomy was in maternal meiosis I in three cases and in maternal meiosis II in two (see table 2).

Discussion

Analysis of SSR DNA polymorphisms was used to determine the mechanism of mosaic trisomy 21. The two main distinct mechanisms examined were the following: In the first (class I), there was a meiotic error that resulted in a trisomy 21; subsequently, there was a mitotic (somatic) loss of one of the three chromosomes 21, resulting in mosaicism for a normal and a trisomic cell line. Such a mechanism is obviously the cause of mosaicism 46,XX/47,XXY, in which there is a meiotic NDJ and postzygotic loss of the Y chromosome (Turpin et al. 1962). The diagnostic hallmark used to assign a case to this mechanism was the detection, in the DNA of the proband, of a third polymorphic allele for a given chromosome 21 polymorphic locus. We were able to detect a "third allele" with more than one polymorphic marker in 10 of the 17 cases examined. In the majority of these cases, the trisomic cell line was present in 6%–15% of the cells examined in the sample from which

DNA was extracted for polymorphism analysis. We therefore conclude that the PCR can reliably detect mosaicism at the 5%–10% level, as was shown previously in one case of mosaicism for trisomy 14 (Antonarakis et al. 1993*b*).

In the second mechanism (class II), the triplication of chromosome 21 is probably the result of a mitotic event similar to that described by Antonarakis et al. (1993*a*). One possibility (mechanism IIA) is that there was a normal zygote (i.e., with two chromosomes 21) that experienced a mitotic (somatic) error that resulted in three chromosomes 21 in some cells. If it is assumed that there was no mitotic recombination, the supernumerary chromosome would be indistinguishable from one of the two chromosomes 21 of the disomic zygote, and therefore analysis of DNA polymorphisms will fail to detect the "third allele," since allele intensity is not sufficient for detection. An alternative mechanism (class IIB) that also fits the data is that of premeiotic duplication of a chromosome 21 (i.e., mosaicism in one of the parents), meiotic segregation in which one gamete had two chromatids 21, trisomic zygote, and finally loss of one of the three chromosomes 21. This class IIB mechanism, although theoretically possible, contains more than one abnormal event and is perhaps less likely than the class IIA mechanism. A total of seven cases examined in this study belong to class II. In case DSmos11, a 1% mosaicism in blood cells has been cytogenetically detected in the father of the mosaic trisomy 21 proband, and therefore the class IIB mechanism is more likely. Familial mosaicism, although rare, has been previously described for aneuploidies of sex chromosomes and autosomes (see, e.g., Juberg et al. 1990). The third potential mechanism (class IIC), in which there is a meiosis II error with lack of crossover in the preceding meiosis I, followed by a mitotic loss after fertilization, is a less likely possibility. The female linkage map of chromosome 21q is $\geq$85 cM (McInnis et al. 1993), and therefore there is, in normal meiosis I, at least one crossover event that should be detectable in meiosis II errors. The genetic length of 21q in male meiosis is $\geq$45 cM (McInnis et al. 1993), and therefore achiasmatic meiosis I may occur only rarely (for further discussion, see Antonarakis et al. 1993).

In the class I mechanism, one expectation is that most ($\sim$95%) of the meiotic errors are maternal in origin. This expectation is based on recent results from studies of >400 cases of free trisomy 21 (Sherman et al. 1991; Antonarakis et al. 1992, 1993*a*; Antonarakis 1993). The results from the 10 cases that belong to class I are compatible with those expected, since 9 of these cases are of maternal origin. The ratio of maternal meiosis I errors to maternal meiosis II errors in the large series of trisomy 21 studies using DNA polymorphism analysis (Sherman et al. 1991; Antonarakis et al. 1992; Antonarakis 1993) was 306:84, or 3.64:1. In the nine maternal cases described in this study, there were six from meiosis I error and three from meiosis II error; the sample size is not sufficient to document that the slight excess of meiosis II errors is statistically significant and of biological importance. It is of interest that this mitotic loss of chromosome 21 often leads to uniparental disomy for chromosome 21 (UPD21) in the euploid cells. This is, for example, the result of the loss of the paternally derived chromosome 21 in a trisomy 21 zygote from maternal meiotic error. UPD21 was observed in the euploid cell lines in cases DSmos6, DSmos12, and DSmos13. It is unknown whether UPD21 alters the phenotype of the individual with mosaic trisomy 21. Creau-Goldberg et al. (1987) and Blouin et al. (1993) did not find any abnormal phenotype in individuals with maternal or paternal UPD21. Another expectation in the class I mechanism is that the mitotic chromosome 21 loss is random; therefore the ratio of maternal chromosome loss to paternal chromosome loss is expected to be 2:1 in mitotic loss that follows maternal meiotic NDJ and vice versa in the mitotic loss that follows paternal meiotic NDJ. The data from the eight cases of maternal meiotic error trisomy 21 that resulted in mosaicism show that the lost chromosome 21 was maternal in five cases and paternal in three. This result is not different from that expected, implying that the parental origin of the lost chromosome 21 is irrelevant to the mechanism of mitotic loss; however, a larger sample is needed for statistical significance. In family DSmos17, the 95% mosaicism for the trisomy 21 cell line did not permit determination of the lost allele, since there was no difference in intensity of the alleles in the autoradiogram. Only one marker, D21S145 (see table 1), showed a consistent intensity difference, which suggested that the lost allele was paternal. However, since only one marker was informative and since this may reflect a peculiarity with this marker rather than a problem with other markers, we did not include this result in the conclusions.

The mean maternal age of the nine cases of class I mechanism was 31.4 years and that of the cases of class II mechanism was 27.4 years. The mean maternal age of the nine cases of class I mechanism is, as expected, not different from the mean maternal age of $\sim$390 cases of trisomy 21 of maternal meiotic origin (Sherman et al. 1991; Antonarakis et al. 1992; Antonarakis 1993). In

addition, the mean maternal age of the seven cases of mitotic duplication of chromosome 21 in class II mechanism was similar to the mean maternal age of 28.5 years for the 11 mitotic cases described by Antonarakis et al. (1993*a*).

The PCR is a powerful method that allows the detection of polymorphic alleles present in a minority of the cells from which DNA has been extracted and used as the template for the reaction. In this study, a "third allele" has been detected in mosaicism ≥6%. Therefore, we might assume that any existing supernumerary allele should have been detected in all cases where its incidence was >6%. The correlation between the intensity of autoradiographic bands observed after PCR and the percentage of trisomic cells from the cytogenetic analysis was far from linear, sometimes showing discrepancy between molecular and cytogenetic data. In DSmos13, with 88% trisomic cells, the allelic "third" band was very close in intensity to the bands corresponding to the other two alleles; in contrast, in DSmos16, with 50% trisomic cells, the bands for the "third" allele were far less than half the intensity of the other two alleles. In the case of DSmos17, with 94% trisomy 21, the PCR amplification was unable to distinguish intensity differences among the three different alleles (except for marker D21S145; see above). It is possible that PCR might have failed to detect "third alleles" in some cases; however, this is unlikely in the majority of cases, since many polymorphic markers were typed for each individual. An additional potential problem for the interpretation of the results is the "hiding" of the "third allele" within the stuttering bands of the polymorphic alleles; it is therefore possible that cases classified as class II may belong to class I. This possibility has been designated with an asterisk in the genotypes of table 1. Since the proband's mosaicism may reveal (i) alleles that are not detected in the father's leukocyte DNA because of potential mosaicism in the latter's DNA or (ii) the absence of paternal alleles because of mitotic (somatic) loss, paternity was verified by the study of non–chromosome 21 polymorphic markers. One case of nonpaternity (DSmos7) was discovered and was eliminated from the analysis. Finally, contamination of template DNA with minute amounts of exogenous DNA would result in the appearance of alleles that could be interpreted as "third alleles" and would lead to misclassification of cases. The study of non–chromosome 21 polymorphic markers helped us clarify potential misinterpretations. In case DSmos3, such a contamination was initially detected. The data of table 1 for this family were collected after acquisition of a second sample. In summary, this study demonstrated that several different mechanisms lead to mosaicism for chromosome 21; in ~60% of the cases, the error leading to trisomy 21 is meiotic, and in the remaining 40% it is probably mitotic.

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References

Antonarakis SE (1993) Human chromosome 21: genome mapping and exploration, circa 1993. Trends Genet 9:142–148

Antonarakis SE, Avramopoulos D, Blouin JL, Talbot CC Jr, Schinzel AA (1993*a*) Mitotic errors in somatic cells cause trisomy 21 in about 4.5% of cases and are not associated with advanced maternal age. Nature Genet 3:146–150

Antonarakis SE, Blouin J-L, Maher J, Avramopoulos D, Thomas G, Talbot CC Jr (1993*b*) Maternal uniparental disomy for human chromosome 14, due to loss of a chromosome 14 from somatic cells with t(13;14) trisomy 14. Am J Hum Genet 52:1145–1152

Antonarakis SE, Down Syndrome Collaborative Group (1991) Paternal origin of the extra chromosome in trisomy 21 as indicated by analysis of DNA polymorphisms. N Engl J Med 324:872–876

Antonarakis SE, Petersen MB, McInnis MG, Adelsberger PA, Schinzel AA, Binkert F, Pangalos C, et al (1992) The meiotic stage of nondisjunction in trisomy 21: determination by using DNA polymorphisms. Am J Hum Genet 50:544–550

Avramopoulos D, Talbot CC, Gerken S, Matsunami N, Lalouel JM, White R, Antonarakis SE (1993) Addition of 7 new highly polymorphic markers to the genetic linkage map of human chromosome 21. In: Delabar JM (ed) Chromosome 21 Workshop #4, Paris, Abstracts, p 3

Blouin J-L, Avramopoulos D, Pangalos C, Antonarakis SE (1993) Normal phenotype with paternal uniparental isodisomy for chromosome 21. Am J Hum Genet 53:1074–1078

Creau-Goldberg N, Gegonee A, Delabar J, Cochet C, Cabanis MO, Stehelin D, Turleau C, et al (1987) Maternal origin of a de novo balanced t(21q21q) identified by ETS2 polymorphism. Hum Genet 76:396–398

Dagna-Bricarelli F, Pierluigi M, Grasso M, Strigini P, Perroni L (1990) Origin of extra chromosome 21 in 343 families: cytogenetic and molecular approaches. Am J Med Genet Suppl 7:129–132

Epstein CJ (1989) Down syndrome (trisomy 21). In: Scriver CR, Beaudet AL, Sly WS, Valle D (eds) The metabolic basis of inherited disease. McGraw-Hill, New York, pp 291–326

Hook EG (1982) Epidemiology of Down syndrome. In: Pueschel SM, Rynders JE (eds) Down syndrome: advances in biomedicine and behavioral sciences. Ware, Cambridge, MA, pp 11–25

Juberg RC, Holliday DJ, Hennessy VS (1990) Familial sex chromosomal mosaicism. Am J Med Genet 37:15–17

Lejeune J, Gautier M, Turpin R (1959) Études des chromosomes somatiques de neuf enfants mongoliens. C R Acad Sci 248:1721–1722

McInnis MG, Chakravarti A, Blaschak J, Petersen MB, Sharma V, Avramopoulos D, Blouin JL, et al (1993) A linkage map of human chromosome 21: 43 PCR markers at average intervals of 2.5 cM. Genomics 16:562–571

Magenis E, Chamberlin J (1981) Parental origin of non disjunction. In: de la Cruz FF, Gerald PS (eds) Trisomy 21 (Down syndrome): research perspectives. University Park Press, Baltimore, pp 77–92

Mikkelsen M (1977) Down's syndrome: cytogenetic epidemiology. Hereditas 86:45–59

Niikawa N, Kajii T (1984) The origin of mosaic Down syndrome: four cases with chromosome markers. Am J Hum Genet 36:123–130

Pangalos CG, Talbot CC Jr, Lewis JG, Adelsberger PA, Petersen MB, Serre J-L, Rethoré M-O, et al (1992) DNA polymorphism analysis in families with recurrence of free trisomy 21. Am J Hum Genet 51:1015–1027

Petersen MB, Frantzen M, Antonarakis SE, Warren AC, Van Broeckhoven C, Chakravarti A, Cox TK, et al (1992) Comparative study of microsatellite and cytogenetic markers for detecting the origin of the nondisjoined chromosome 21 in Down syndrome. Am J Hum Genet 51:516–525

Petersen MB, Schinzel AA, Binkert F, Tranebjaerg L, Mikkelsen M, Collins FA, Economou EP, et al (1991) Use of short sequence repeat DNA polymorphisms after PCR amplification to detect the parental origin of the additional chromosome in Down syndrome. Am J Hum Genet 48:65–71

Richards BW (1969) Mosaic mongolism. J Ment Defic Res 13:66–83

——— (1974) Investigation of 142 mosaic mongols and mosaic parents of mongols: cytogenetic analysis and maternal age at birth. J Ment Defic Res 18:199–208

Saiki RK, Scharf S, Faloona F, Mullis KB, Horn GT, Erlich HA, Arnheim N (1985) Enzymatic amplification of beta-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. Science 230:1350–1354

Sherman SL, Takaesu N, Freeman SB, Grantham M, Phillips C, Blackston RD, Jacobs PA, et al (1991) Trisomy 21: association between reduced recombination and nondisjunction. Am J Hum Genet 49:608–620

Taylor AI (1968) Cell selection in vivo in normal/G trisomic mosaics. Nature 219:1028–1030

——— (1970) Further observations of cell selection in vivo in normal/G trisomic mosaics. Nature 227:163–165

Turpin R, Lejeune J, Breton A (1962) Hermaphroditism XX/XXY. C R Acad Sci 255:3088

Weber JL, May PE (1989) Abundant class of human DNA polymorphisms which can be typed using the polymerase chain reaction. Am J Hum Genet 44:388–396