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Comparative Study of Microsatellite and Cytogenetic Markers for Detecting the Origin of the Nondisjoined Chromosome 21 in Down Syndrome

Michael B. Petersen,* Merete Frantzen,* Stylianos E. Antonarakis,† Andrew C. Warren,† Christine Van Broeckhoven,‡ Aravinda Chakravarti,§ Tara K. Cox,§ Connie Lund,* Bodil Olsen,* Hanne Poulsen,* Annie Sand,* Niels Tommerup,* and Margareta Mikkelsen*

*Department of Medical Genetics, John F. Kennedy Institute, and Danish Centre for Human Genome Research, Glostrup, Denmark; †Center for Medical Genetics, Department of Pediatrics, Johns Hopkins University School of Medicine, Baltimore; ‡Department of Biochemistry, University of Antwerp, Antwerp; and §Department of Human Genetics, University of Pittsburgh, Pittsburgh

Summary

Nondisjunction in trisomy 21 has traditionally been studied by cytogenetic heteromorphisms. Those studies assumed no crossing-over on the short arm of chromosome 21. Recently, increased accuracy of detection of the origin of nondisjunction has been demonstrated by DNA polymorphism analysis. We describe a comparative study of cytogenetic heteromorphisms and seven PCR-based DNA polymorphisms for detecting the origin of the additional chromosome 21 in 68 cases of Down syndrome. The polymorphisms studied were the highly informative microsatellites at loci D21S215, D21S120, D21S192, IFNAR, D21S156, HMG14, and D21S171. The meiotic stage of nondisjunction was assigned on the basis of the pericentromeric markers D21S215, D21S120, and D21S192. Only unequivocal cytogenetic results were compared with the results of the DNA analysis. The parental and meiotic division origin could be determined in 51% of the cases by using the cytogenetic markers and in 88% of the cases by using the DNA markers. Although there were no discrepancies between the two scoring systems regarding parental origin, there were eight discrepancies regarding meiotic stage of nondisjunction. Our results raise the possibility of recombination between the two marker systems, particularly on the short arm.

Introduction

Trisomy 21 (Down syndrome), with an incidence of 1:1,100, is the most common genetic cause of mental retardation, whereas fragile-X syndrome is the most common inherited form of mental retardation, with an incidence of about 1:1,200 boys and 1:2,000 girls. Trisomy 21 is usually due to meiotic nondisjunction. Several studies have been carried out to determine the meiotic origin of the additional chromosome 21 in Down syndrome. For more than 20 years, cytogenetic heteromorphisms on chromosome 21 have been used for nondisjunction studies (reviewed by del Mazo et al. 1982; Juberg and Mowrey 1983; Hassold and Jacobs 1984; Bricarelli et al. 1989). Those studies assumed no crossing-over on the short arm, for the assignment of meiotic stage of nondisjunction, except in one study where this possibility was suggested (Mikkelsen et al. 1980). Since 1984, chromosome 21–specific DNA probes have been used in the analysis of nondisjunction by DNA polymorphism analysis (Davies et al. 1984; Antonarakis et al. 1985; Stewart et al. 1985; Millington-Ward and Pearson 1988; Rudd et al. 1988; Galt et al. 1989). A few studies have used cytogenetic heteromorphisms in combination with DNA polymorphisms in a limited number of families (Bricarelli et al. 1988, 1990; Stewart et al. 1988; Meijer et al. 1989; Hamers et al. 1990; Perroni et al. 1990).

Within the last year, two large collaborative studies, one of 200 and the other of 104 trisomy 21 families, respectively, have used multiple DNA polymorphisms

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Address for correspondence and reprints: Michael B. Petersen, M.D., Ph.D., Department of Medical Genetics, John F. Kennedy Institute, 7 Gl. Landevej, DK-2600 Glostrup, Denmark.

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spanning the long arm of human chromosome 21, to determine the origin of nondisjunction (Antonarakis et al. 1991; Sherman et al. 1991). The DNA studies estimated that a higher proportion of the cases had maternal origin, as compared with the cytogenetic studies: 95% from DNA studies versus 80% from cytogenetic studies. This difference was attributed to an increased accuracy of the DNA polymorphism analysis, as demonstrated by erroneous cytogenetic determinations in a subgroup of families (Antonarakis et al. 1991; Sherman et al. 1991).

We have recently demonstrated the usefulness of highly polymorphic microsatellite DNA polymorphisms for the study of nondisjunction in Down syndrome (Petersen et al. 1991*a*). In the present paper we present a comparative study between seven PCR-based DNA markers and cytogenetic heteromorphisms for the detection of the origin of the nondisjoined chromosome 21 in 68 Down syndrome families. The study is therefore also a test of the null hypothesis—that there is no crossing-over between the two marker systems. No discrepancies on parental origin were found, but 8/38 maternal cases were discrepant for meiotic stage of nondisjunction. This provides some evidence for recombination between the chromosome 21 short arm markers and the most proximal long arm DNA markers.

Subjects and Methods

Patient Population

The patient population consisted of 68 trisomy 21 probands and their parents; all but two were diagnosed at The John F. Kennedy Institute, Denmark, within the years 1983–91 and were selected only on the availability of DNA samples from the proband and both parents. Two cases were referred from Norway: the occurrence of trisomy 21 in two probands, the fathers of whom were brothers. Seventeen of the cases were part of other studies (Antonarakis et al. 1991; Sherman et al. 1991). The 68 probands consisted of 52 newborns and 16 prenatal cases. The karyotypes of the probands were 47,XY,+21 (35 cases), 47,XX,+21 (29 cases), 46,XY/47,XY,+21 (2 cases; twins), and one each of 46,XX/47,XX,+21 and 46,XX,t(11;12)pat/47,XX,t(11;12)pat,+21.

Cytogenetic Analysis

The chromosome 21 short arm heteromorphisms were studied by QFQ banding and subsequent Ag-NOR staining of chromosomes from blood lymphocyte cultures (Mikkelsen et al. 1980). The assignment of meiotic stage of nondisjunction was based on the assumption that no crossing-over occurs between the heteromorphic areas and the centromere. All cases were examined using both direct microscopy and photographic analysis. Only unequivocal results were compared with the results of the DNA analysis. The chromosomal and DNA polymorphisms of all cases were examined by at least two observers and were scored blindly, by different observers, and later were compared.

DNA Analysis

The DNA polymorphisms studied were seven microsatellite DNA polymorphisms due to dinucleotide repeats (Litt and Luty 1989; Smeets et al. 1989; Tautz 1989; Weber and May 1989) or to a variable poly(A) tract of an Alu repetitive element (AluVpA) (Economou et al. 1990; Zuliani and Hobbs 1990) and were detected after PCR amplification of genomic DNA (Saiki et al. 1985). The polymorphisms studied were (1) a $(CA)_n$ repeat at D21S215 (Warren et al., in press), (2) a $(GT)_n$ repeat at D21S120 (Burmeister et al. 1990), (3) a $(CA)_n$ repeat at D21S192 (Van Camp et al. 1991), (4) an $(ATTT)_n$ repeat of an AluVpA within IVS5 of the IFNAR gene (McInnis et al. 1991), (5) a $(GT)_n$ repeat at D21S156 (Lewis et al. 1990), (6) a $(GT)_n$ repeat within IVS5 of the HMG14 gene (Petersen et al. 1991*a*), and (7) an $(AC)_n$ repeat at D21S171 (Petersen et al. 1991*c*). PCR amplification was performed in a total volume of 22 μl containing 1 μg of genomic DNA; 57 μM each of dATP, dCTP, dGTP, and dTTP; 0.2 μM of each of the primers (the one end-labeled with [γ-^{32}P]ATP); 0.8 units of *Taq* DNA polymerase (Boehringer); 10 mM Tris-HCl (pH 8.3); 1.5 mM $MgCl_2$; 50 mM KCl; and 0.1 mg gelatin/ml. End-labeling of primers was performed at 37°C in a 50 μl reaction volume containing 5 μM primer, 100 μCi [γ-^{32}P]ATP at 6,000 Ci/mmol, 50 mM imidazole-HCl (pH 6.4), 12 mM $MgCl_2$, 15 mM 2-mercaptoethanol, 0.3 mM ADP, 0.5 μM ATP, and 20 units T4 polynucleotide kinase (Gibco BRL). PAGE of the amplification products and autoradiography were performed according to the protocols described elsewhere (Economou et al. 1990; Petersen et al. 1990). The primers used and the PCR conditions for loci D21S215, D21S120, D21S192, IFNAR, D21S156, and HMG14 have been described elsewhere (Burmeister et al. 1990; Lewis et al. 1990; McInnis et al. 1991; Petersen et al. 1991*a*; Van Camp

et al. 1991; Warren et al., in press). For locus D21S171, a new set of primers was synthesized from the sequence flanking the $(AC)_n$ repeat of clone Mfd95 (courtesy of Dr. J. L. Weber) in order to obtain a bigger PCR product, with fewer nonspecific bands, than has been previously published (Petersen et al. 1991*c*). The new primers were primer 1—5′GATCAAGTTAAGAGGAGGCT 3′—and primer 2—5′GTGGGCTGCTGCTATCTTTG 3′—with an expected product size of 204 bp. Primer 2 was end labeled, and the PCR conditions were as described by Petersen et al. (1991*c*), except that the annealing temperature was 58°C.

The seven DNA polymorphisms studied are highly informative markers on 21q, with 6–12 alleles and with observed heterozygosity of 54%–83% (see references cited above). All loci have been localized on the genetic linkage map of human chromosome 21 (Petersen et al. 1991*b*), on the basis of data from the 40 CEPH (Centre d'Etude du Polymorphisme Humain) families (Dausset et al. 1990). The markers D21S215, D21S120, and D21S192 are located in chromosomal bands q11.1-q11.2 and are 0, 6, and 7 cM, respectively, from an alphoid DNA polymorphism specific for the human chromosome 21 centromere (Burmeister et al. 1990; Jabs et al. 1991; Van Camp et al. 1991; Antonarakis et al. 1992; Warren et al., in press; authors' unpublished data). The IFNAR gene has been localized to q22.1 (McInnis et al. 1991), while D21S156, HMG14, and D21S171 loci were mapped to the terminal band q22.3 (Lewis et al. 1990; Petersen et al. 1990, 1991*c*). The physical location and the genetic distances of the markers studied are shown in figure 1.

The parental origin of the extra chromosome 21 was determined by dosage analysis, when two different alleles were present in the proband (the validity of the method was demonstrated in Petersen et al. 1991*a*), or by scoring the polymorphic alleles, when three different alleles were present in the proband (Petersen et al. 1991*a*). The meiotic stage of nondisjunction (meiosis I or II) was assigned on the basis of reduction to homozygosity at the pericentromeric 21q markers D21S215, D21S120, and D21S192 by using the methods of Chakravarti and Slaugenhaupt (1987) and Chakravarti (1989).

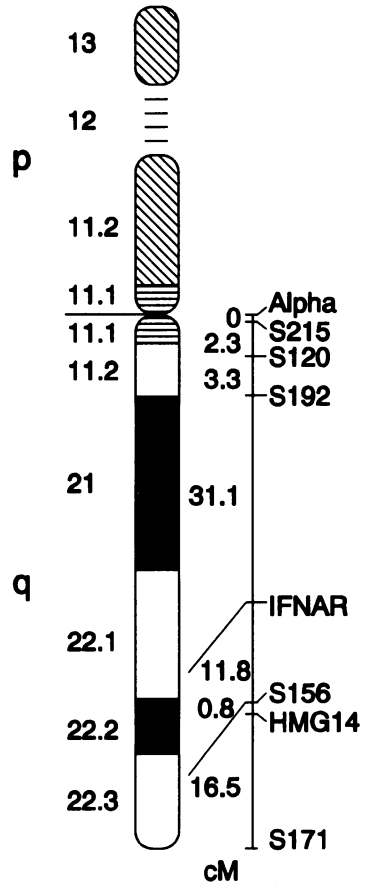

Figure 1 Chromosome 21 ideogram showing approximate physical location of the DNA loci studied. The numbers to the left of the chromosome indicate chromosomal bands. The numbers to the right indicate the estimated sex average genetic distances between the markers. Alpha = alpha satellite DNA polymorphism D21Z1 (not studied); S215 = D21S215; S120 = D21S120; S192 = D21S192; S156 = D21S156; and S171 = D21S171.

Results

The parental and meiotic division origin of the additional chromosome 21 could be determined in 51% of the cases by using the chromosomal markers and in 88% of the cases by using the DNA markers (table 1). In 52 cases, at least two DNA markers were informative regarding the parental origin, and no inconsistencies were found between these markers. In the 34 cases where more than one of the pericentromeric markers (D21S215, D21S120, and D21S192) were informative on reduction/nonreduction to homozygosity, there was no recombination observed between those markers.

There were no discrepancies on parental origin between the cytogenetic heteromorphisms and the DNA polymorphisms in 33 cases, where both systems were informative. However, there were eight discrepancies on meiotic stage of nondisjunction of 38 maternal

Table 1

Parental Origin and Meiotic Stage of Nondisjunction in 68 Cases of Trisomy 21, Using Cytogenetic Heteromorphisms Compared with DNA Polymorphisms

A. Parental Origins			
Determination by	No. of Cases (%)	Paternal	Maternal
Cytogenic heteromorphisms	37 (54)	2	35
DNA polymorphisms	63 (93)	5	58

B. Parental and Meiotic Origins					
		No. of Division Errors			
Determination by	No. of Cases (%)	Paternal I	Paternal II	Maternal I	Maternal II
Cytogenetic heteromorphisms	35 (51)	1	1	25	8
DNA polymorphisms	60 (88)	1	3	40	16

cases, where both systems were informative (table 2). Of these eight discrepant cases, the DNA markers consistently showed a meiosis II error, and the cytogenetic markers showed a meiosis I error in seven cases; in one case, the DNA markers showed a meiosis I error, and the cytogenetic markers showed a meiosis II error (table 2). The genotypes of the chromosomal heteromorphisms and the DNA polymorphisms in the eight discrepant families are given in table 3, and examples of discrepant families are shown in figure 2. In the four mosaic cases the DNA markers showed a maternal I error in one case (meiosis I error by cytogenetic markers) and were uninformative in three cases where the cytogenetic markers showed maternal II errors (or mitotic errors).

Discussion

The usefulness of PCR-based highly informative microsatellite DNA polymorphisms for the study of nondisjunction in Down syndrome has recently been demonstrated (Petersen et al. 1991*a*). In a study of 87 families with Down syndrome, the parental origin of the additional chromosome 21 was determined in 68 cases (78%) by using two loci (D21S156 and HMG14) on human chromosome 21 (Petersen et al. 1991*a*). In the present study of 68 trisomy 21 cases, the parental origin could be determined in 63 cases (93%) by using seven different highly polymorphic PCR markers on 21q. This is comparable to the 96% success rate of the two large collaborative studies (Antonarakis et al. 1991; Sherman et al. 1991) using 15 or more DNA polymorphisms, the vast majority of which were studied by Southern (1975) blot analysis. Theoretical calculations have also demonstrated the necessity of using multiple genetic markers to identify the parental origin, particularly by using multiallelic polymorphisms (Chakravarti 1989).

The reliability of cytogenetic heteromorphisms in determining the parental origin of nondisjunction in trisomy 21 has been recently disputed (Antonarakis et al. 1991; Sherman et al. 1991). In a subgroup of families where cytogenetic and DNA polymorphic markers were compared, discrepant cytogenetic determinations were made in 3/31 cases (Antonarakis et al. 1991) and 2/25 cases (Sherman et al. 1991). This discrepancy was explained by the more subjective nature of the cytogenetic scoring of chromosomal variants as compared with DNA polymorphism analysis, which can be verified with several markers. By using only unequivocal chromosomal heteromorphisms in the present study, we have assigned the parental origin in 54% of cases, which is obviously less than the pre-

Table 2

Distribution of the Assignment of Meiotic Stage of Nondisjunction in Maternal Trisomy 21 Cases, Using Cytogenetic Heteromorphisms Compared with DNA Polymorphisms

DNA Polymorphisms in	Assignment by Cytogenetic Heteromorphisms	
	Meiosis I	Meiosis II
Meiosis I	26	1
Meiosis II	7	4

Table 3

Chromosomal Heteromorphisms and DNA Polymorphisms in Trisomy 21 Families: Discrepancies between the Two Scoring Systems Regarding the Assignment of Meiotic Stage of Nondisjunction

	Genotype for Case Numbers[b]															
Locus[a]	DS065			DS068			DS083			DS095			DS104			DS110			DS197			DS230		
Cyto	ab-abe-bc[c]		NR	ab-acd-cd	M	NR	ab-abc-bc		NR	ab-bcd-cd	M	NR	ab-bcd-cd	M	NR	ab-bcd-cd	M	NR	ab-bcd-cd	M	NR	ab-add-cd	M	R
D21S215	22-112-12	M	R	11-111-12		R	11-111-12		R	11-122-12	M	R	22-112-12	M	R				23-223-12		R	22-123-13	M	NR
D21S120	12-111-12		R	12-122-12			11-133-23	M	R	11-111-12		R	23-112-14	M	R	12-233-23	M	R	11-111-12		R	12-122-22		
D21S192	11-122-12	M	R	11-111-11			11-111-12		R	12-111-12		R	11-133-23	M	R	12-122-12						11-111-11		
IFNAR ...	23-122-12		NR	13-123-23		NR	13-233-23		NR	13-123-23		NR	13-122-22	M		12-123-23		NR	12-222-23		R	13-112-12		NR
D21S156	14-123-23	M	NR	34-124-12	M	NR	22-123-13	M	NR	34-124-12	M	NR	12-123-23		NR	12-122-12			12-244-34	M	R	12-234-34	M	NR
HMG14	13-124-24	M	NR	34-123-12	M	NR	24-123-13	M	NR	34-123-12	M	NR	11-112-12		NR	14-234-23	M	NR	11-111-11					
D21S171	33-123-12	M	NR	11-112-12		NR	23-123-13		NR	12-233-23		NR	12-113-13		NR	11-112-12		NR	11-111-11			34-124-12	M	NR

[a] Cyto = cytogenetic heteromorphisms on the short arm of chromosome 21. The other chromosome 21 DNA loci are ordered in rows corresponding to their relative chromosomal order on the long arm, based on linkage analysis. D21S215 is the closest to the centromere of chromosome 21, and D21S171 is the closest to the telomere of the long arm of human chromosome 21.

[b] The three parts of the data correspond to the genotypes of the father, proband, and mother, respectively. The letters in the cytogenetic genotypes represent the different short arm heteromorphisms. The numbers in the DNA genotypes represent the different alleles at a specific locus. M = maternal origin of the additional chromosome 21, as determined by the respective cytogenetic or DNA marker. R and NR = reduction to homozygosity or nonreduction to homozygosity, respectively, for a given marker (for details, see text).

[c] The proband showed three different short arm markers, one of which (e) was not found in the parents (see Discussion).

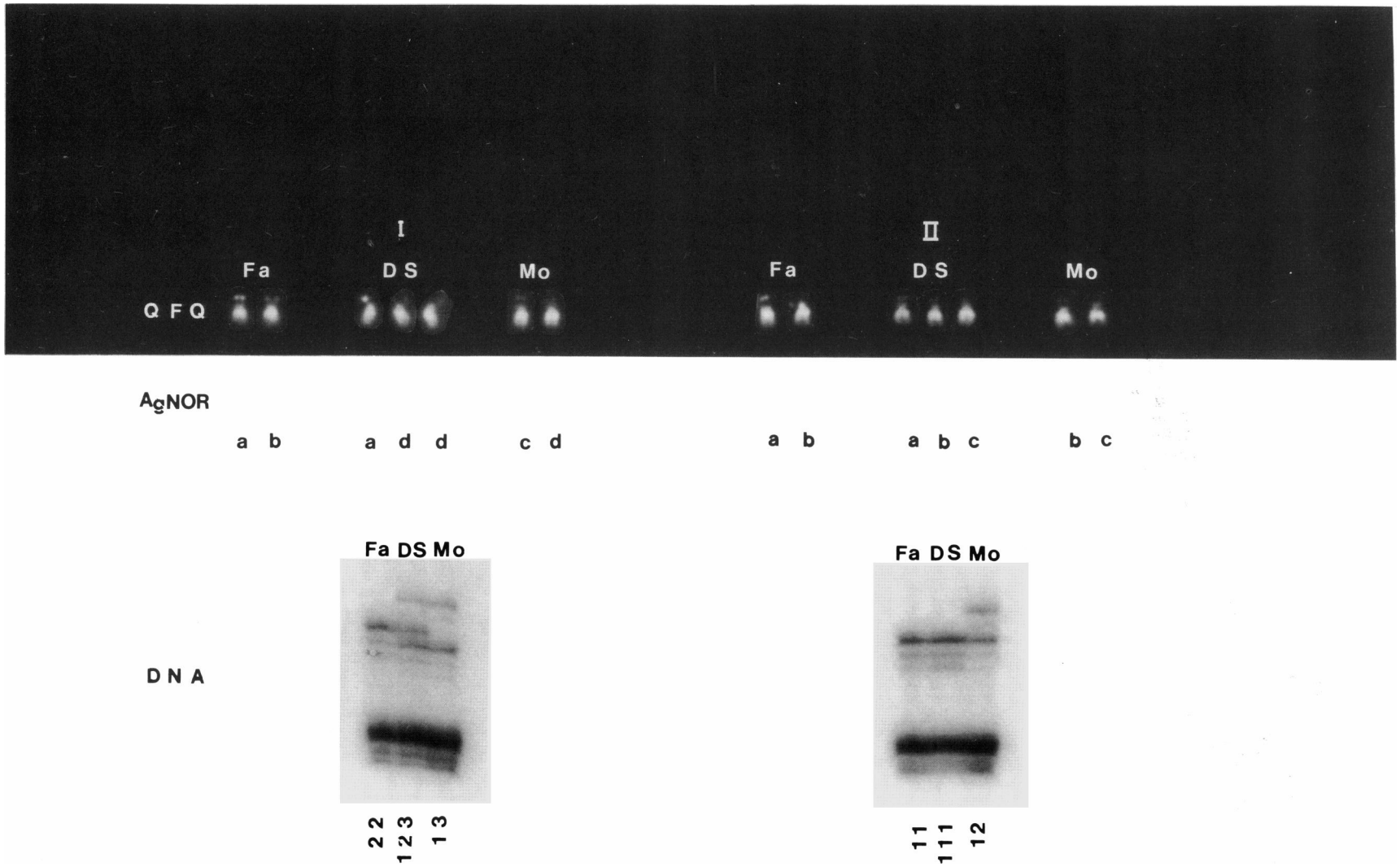

Figure 2 Determination of origin of additional chromosome 21 in two nuclear families with trisomy 21, where cytogenetic heteromorphisms and DNA polymorphisms were discrepant regarding meiotic stage of nondisjunction (I = case DS230 of table 3; and II = case DS083 of table 3). *Top*, Chromosomal heteromorphisms studied by QFQ banding and AgNOR staining. *Bottom*, Dinucleotide repeat at D21S215, which was the DNA polymorphism closest to the centromere studied and which shows a lower constant band. The genotypic scoring of heteromorphisms and alleles is indicated for each individual. Fa = father; DS = Down syndrome proband; and Mo = mother.

viously reported 76% (Mikkelsen et al. 1980), but there are no discrepancies, in comparison with the DNA polymorphism analysis.

The study of meiotic stage of nondisjunction in trisomy 21 by using DNA polymorphisms has been hampered by the lack of centromeric markers. Recently, however, alphoid DNA polymorphisms specific for the human chromosome 21 centromere have been described (Jabs et al. 1991). The informativeness of these chromosome 21–specific alphoid markers is low, and they are not useful for routine nondisjunction studies. However, the alphoid DNA polymorphisms have been localized on the genetic linkage map of human chromosome 21 (Jabs et al. 1991) on the basis of data from the 40 CEPH families (Dausset et al. 1990), giving an estimate of the genetic distance between the centromere and the closest pericentromeric markers on the long arm of chromosome 21.

The use of pericentromeric DNA polymorphic markers on chromosome 21 to determine the meiotic stage of nondisjunction in 200 families with free trisomy 21 was presented in a recent study (Antonarakis et al. 1992). By using the polymorphic markers D21S120, D21S16, D21S13, and D21S172 (considered as one locus at a distance of 6 cM from the centromere), the meiotic-division error (and parental origin) was determined in 175 families (87%) (Antonarakis et al. 1992). In the determination of the meiotic stage of nondisjunction, a comparison of the cytogenetic and DNA polymorphic markers was performed in a subset of 31 families from three different cytogenetic laboratories, and three discrepancies were found (Antonarakis et al. 1992).

In the present study using three highly informative dinucleotide repeat markers at the pericentromeric loci D21S215, D21S120, and D21S192, we found eight discrepancies among 38 maternal cases informative with both cytogenetic and DNA polymorphism analysis (table 2). These discrepancies could be due to errors in either of the two scoring systems. Since only unequivocal cytogenetic determinations were compared with the DNA data, and because of the objective nature of the DNA polymorphisms, we suggest that the discrepancies represent meiotic crossing-over between the two marker systems, i.e., between the short arm heteromorphisms (located at the short arm, stalk, or satellite) and the long arm DNA markers. Obviously crossing-over occurs between D21S120/D21S192 and the centromere in normal meiosis, based on the CEPH genetic linkage map. Meiosis leading to nondisjunction has, however, been associated with reduced recombination on the nondisjoined chromosomes in trisomy 21 (chromosome 21 long arm reduced recombination) (Warren et al. 1987; Sherman et al. 1991) and in 47,XXY (pseudoautosomal region–reduced recombination) (Hassold et al. 1991). It is interesting that seven of the eight discrepancies in our study were in the same category (table 2). If we assign the meiotic stage of nondisjunction on the basis of the pericentromeric DNA polymorphisms (table 2), short arm recombination occurred in 1/27 cases among meiosis I errors and in 7/11 cases among meiosis II errors. These data can be explained by reduced (or absent) recombination on the short arm in meiosis I nondisjunction, as has been previously shown for the long arm (Warren et al. 1987; Sherman et al. 1991). This hypothesis can explain the higher frequency of meiosis II errors reported among maternal cases determined by pericentromeric DNA polymorphisms, as compared with those in the cytogenetic studies (Antonarakis et al. 1992). The second possibility is that the short arm heteromorphisms actually represent the centromere and that the discrepancies are due to crossing-over between the centromere and the DNA polymorphisms on the proximal long arm. Preliminary results from XXX and XXY trisomies of maternal origin have suggested an excess recombination in the pericentric region of the X chromosome (Morton et al. 1990). Whether there exists a causal relationship between pericentromeric increased recombination and nondisjunction in trisomy 21 cannot be elucidated until a centromere-specific chromosome 21 marker has been identified.

One family (family DS065 in table 3) in the present study clearly showed three different chromosome 21 cytogenetic heteromorphisms in the proband, one of which was not detected in the parents. The DNA polymorphism analysis showed maternal origin of the additional chromosome, with pericentromeric markers indicating a second division error. Possible explanations include maternal mosaicism (Nielsen et al. 1988), "jumping satellites" (exchange between the short arms of acrocentric chromosomes in maternal meiosis or in the proband), or meiotic, unequal short arm crossing-over in the mother. The last alternative seems the most likely in light of our data and since no new alleles were detected in the proband, with any of the multiallelic DNA markers studied.

Very little is known regarding short arm recombination on the acrocentric chromosomes (Mikkelsen et al. 1980). No short arm polymorphism has so far been included in the genetic linkage map of human chromo-

some 21. A mean chiasma frequency of .01 on the chromosome 21 short arm was estimated from meiotic chiasma counts in seven karyotypically normal although infertile males (Laurie and Hultén 1985). It would thus be of interest to estimate the genetic distance between the short arm heteromorphisms and the pericentromeric DNA polymorphisms by analyzing cell lines from the CEPH families. This would give information about chromosome 21 short arm recombination and about the reliability that the short arm heteromorphisms have in the determination of the meiotic stage of nondisjunction in trisomy 21. The study will be impossible if exchange of short arm material occurs among other acrocentric chromosomes in meiosis.

The isolation of more DNA polymorphisms, both centromeric and pericentromeric (including the short arm), on human chromosome 21 is needed in order to definitely determine the meiotic stage of nondisjunction in trisomy 21, to examine the altered recombination associated with nondisjunction, to address the question of advanced maternal age, and finally, in combination with epidemiological studies, to examine risk factors for meiotic nondisjunction.

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