Am. J. Hum. Genet. 42:227–236, 1988

Trisomy 21 (Down Syndrome): Studying Nondisjunction and Meiotic Recombination by Using Cytogenetic and Molecular Polymorphisms That Span Chromosome 21

Gordon D. Stewart,* Terry J. Hassold,† Andrew Berg,‡ Paul Watkins,§ Rudolph Tanzi,‡ and David M. Kurnit*

*Howard Hughes Medical Institute, University of Michigan Medical Center, Ann Arbor; †Department of Pediatrics, Cornell Medical School, New York; ‡Genetics Division, The Children's Hospital, Boston; and §Integrated Genetics, Framingham, MA

Summary

By combining molecular and cytogenetic techniques, we demonstrated the feasibility and desirability of a comprehensive approach to analysis of nondisjunction for chromosome 21. We analyzed the parental origin and stage of meiotic errors resulting in trisomy 21 in each of five families by successfully using cytogenetic heteromorphisms and DNA polymorphisms. The 16 DNA fragments used to detect polymorphisms spanned the length of the long arm and detected recombinational events on nondisjoined chromosomes in both maternal meiosis I and maternal meiosis II errors. The meiotic stage at which errors occurred was determined by sandwiching the centromere between cytogenetic heteromorphisms on 21p and an informative haplotype constructed using two polymorphic DNA probes that map to 21q just below the centromere. This study illustrates the necessity of combining cytogenetic polymorphisms on 21p with DNA polymorphisms spanning 21q to determine (1) the source and stage of meiotic errors that lead to trisomy 21 and (2) whether an association exists between nondisjunction and meiotic recombination.

Introduction

During the past 15 years cytogeneticists have used chromosomal variants (heteromorphisms) to study the parental origin of the extra chromosome in trisomy 21. To date, this technique has been used to detail the segregation of chromosome 21 in more than 1,000 Down syndrome families (Bott et al. 1975; Wagenbichler et al. 1976; Magenis et al. 1977; Hansson and Mikkelsen 1978; Mattei et al. 1980; Mikkelsen et al. 1980; Roberts and Callow 1980; Houghton 1981; Jacobs and Mayer 1981; del Mazo et al. 1982; Juberg and Mowrey 1983; Aymé et al. 1986) These studies concluded (1) that most cases result from nondisjunction at maternal meiosis I but (2) that errors at paternal meiosis I and II and maternal meiosis II also occur and account for approximately one-third of all cases. Both conclusions held in virtually all studies and applied to spontaneously aborted, as well as liveborn, conceptuses with trisomy 21. (Hassold et al. 1984)

These studies also raised or left unanswered several other questions concerning the origin of trisomy 21. Two of the most intriguing questions are the following:

1. The maternal age conundrum: Is the maternal age effect due to increased production of abnormal eggs or decreased destruction of abnormal embryos? The association between increasing maternal age and the occurrence of trisomy 21 has been recognized for >50 years (Penrose 1933). The obvious hypothesis for the age effect is the "older-egg" model—that is, that most errors involving the numerical assortment of chromosome 21 occur in oocytes, with the error rate increasing as women age. However, the results of the cytogenetic studies indicate that ~70%–75% of nondisjunctional events involving chromosome 21

Received June 8, 1987; revision received August 20, 1987.

Address for correspondence and reprints: Dr. Gordon Stewart, Howard Hughes Medical Institute, 1150 West Medical Center Drive, MSRB I Room 3520, Ann Arbor, MI 48109-0650.

0002-9297/88/4202-0003$02.00

occur in maternal meiosis I, regardless of maternal age. In contrast, the older-egg model predicts that the proportion of maternal meiosis I errors should increase significantly with advancing maternal age.

This contradiction led, in part, to the "relaxed-selection" hypothesis, which suggests that the age-related increase in frequency of Down syndrome may ensue from an inability of older mothers to reject trisomy-21 conceptuses (Aymé and Lippman-Hand 1982; Stein et al. 1986). This implies that younger mothers have an ability, diminishing with increasing maternal age, to discriminate against chromosomally abnormal conceptuses. Indeed, maternal genotype can affect in utero survival of aneusomic murine conceptuses (Vekemans and Trasler 1985; Biddle et al. 1986).

The relevance of these observations to relaxed selection in humans is unclear. There is evidence against the model: Down syndrome resulting from an unbalanced translocation does not show significant maternal age dependence, as would be predicted on the basis of the hypothesis (Hook 1983). Further, the relaxed-selection model depends entirely on analysis of chromosomal heteromorphisms, a technique that has at least three limitations. First, the heteromorphisms on 21p are only on one side of the centromere, so that crossovers between the centromere and the 21p marker remain undetected and confound attempts to determine whether nondisjunction occurred at either the first or second divisions. Second, cytogenetic heteromorphisms are uninformative in a substantial fraction of families. Third, the analysis is based on subjective evaluation of the size and staining quality of the variants and may be subject to observer bias or experimental error. This last point is particularly relevant, since Carothers (1987) has demonstrated that in cytogenetic determinations of parental origin an error rate as low as 8% is sufficient to reconcile the published cytogenetic observations with an older-egg model.

Therefore, the intuitively appealing hypothesis that advanced maternal age is associated with increased nondisjunction and not with decreased selection remains viable. The definitive study to resolve this issue will require virtually complete ascertainment of the parental source of trisomy in a large number of Down syndrome subjects. The present, pilot study documents the feasibility of combining analysis of cytogenetic markers with analysis of DNA-based polymorphisms to achieve this goal.

2. Is there a correlation between crossing-over and nondisjunction on chromosomes 21? Since bivalents are held together during meiosis I by their chiasmata, a reduction in chiasma number may predispose to univalent formation and, ultimately, to nondisjunction. In the mouse, this has been suggested to be the basis for age-related nondisjunction. For example, Henderson and Edwards (1968) observed declining chiasma frequencies and increased incidence of univalents in aging mouse oocytes and suggested that age-dependent trisomy results from random segregation of univalents. Since chiasma formation occurs prenatally in the female, these investigators elaborated "a production-line" model in which chiasma frequency was decreased in oocytes formed later during development, with the oocytes being ovulated in the same order as they entered meiosis. Subsequently, several studies have confirmed the age-related decrease in chiasmata and increase in univalents in the female mouse (Luthardt et al. 1973; Polani and Jagiello 1976; Speed 1977), hamster (Sugawara and Mikamo 1983), and human (Luthardt 1977); and Jagiello and Fang (1979) reported a lower chiasma frequency among mouse oocytes entering diplotene on day 18 than among oocytes entering it on day 16 of gestation. However, Speed and Chandley (1983) were unable to confirm Jagiello and Fang's observation, and several studies of aging female mice and hamsters did not find a correlation between univalent formation at meiosis I and aneusomy either later in meiosis or in early embryonic stages (Henderson and Edwards 1968; Polani and Jagiello 1976). Thus, the validity of the model, as well as the contribution of chiasmata loss to age-dependent trisomy, remains unproved.

Very few data are available from humans on the possible relationship between chiasma frequency and nondisjunction. On the basis of data from spontaneous abortions, Hassold et al. (1980*b*) suggested that heterogeneous maternal age effects among autosomal trisomies could reflect variation in chiasma frequency among chromosomes. If maternal age–related nondisjunction ensued from loss of chiasmata with age, the effect should be most pronounced among the smallest chromosomes, i.e., those with the fewest chiasmata. Indeed, an inverse correlation exists between the estimated number of chiasmata for a particular chromosome (Laurie and Hultén 1985) and the mean maternal age of trisomy for that chromosome (Hassold et al. 1980*b*, 1984).

DNA polymorphisms can also be used to study the parental origin of the extra chromosome in trisomy 21 (Davies et al. 1984; Stewart et al. 1985). In a study of 34 families, Antonarakis et al. (1986) recently provided the first direct evidence suggesting that recombination is depressed on the chromosome 21 involved in nondisjunction. Because of the small number of families sampled and the absence of DNA markers from the distal portion of the linkage map on 21q (Tanzi et al., submitted), this study needs to be repeated with a larger number of families and a larger number of probes that span the long arm of chromosome 21. Emphasis for the latter point comes from the linkage study of Tanzi et al. (submitted), which demonstrates a high frequency of terminal chiasmata on 21q. Further, an essential feature of this type of analysis is that markers tightly linked to the centromere are used to determine the meiotic stage of nondisjunction, allowing the determination of whether altered recombination is associated with a particular class of meiotic errors. Below we describe a pilot study that demonstrates the feasibility of combining molecular and cytogenetic analyses to determine the interrelationships between nondisjunction and recombination. An expansion of this pilot study will permit the investigation of whether altered recombination on all or part of chromosome 21 is associated with nondisjunction.

Material and Methods

Cytogenetic Analysis

The five trisomy-21 samples documented in the present report were ascertained in a cytogenetic study of spontaneous abortions (Hassold et al. 1980*a*). Four of the five were single trisomies, and one was trisomic for chromosome 7 as well as for chromosome 21.

Chromosomal preparations from parental lymphocytes were compared with those from the abortuses by using Q-banding with dichloromethoxyacridine/spermine (Degau et al. 1978). In some cases the slides had already been silver stained for analysis of nucleolar-organizing-region (NOR) variants before being Q-banded, making it possible to examine the NOR and Q-heteromorphisms simultaneously (Hassold et al. 1987). All cases were examined directly on the microscope by at least two independent observers, and, in the event of a disagreement, the final decision was the most conservative one compatible with both sets of observations.

Molecular Analysis

DNA was prepared from blood by means of the method of Kunkel et al. (1977). DNA was digested with the appropriate restriction enzyme (New England Biolabs) and fractionated by electrophoresis in 0.8% SeaKem agarose. The methods used for transfer to Genatran filters (Plasco, Inc.) and protocols for hybridization have been described elsewhere (Neve et al. 1986). The single-copy probes used in the present study (Lieman-Hurwitz et al. 1982; Stewart et al. 1985; Watkins et al. 1986; G. D. Stewart, U. Tantravahi, and D. M. Kurnit, unpublished data) are displayed in figure 1 and table 1. DNA probes were radiolabeled by means of the random-primer method of Feinberg and Vogelstein (1983).

We used a recombination-based assay (Neve and Kurnit 1983; Seed 1983) to detect a short region of DNA sequence homology, shared by 724 family members, on the short arm of chromosome 21 (Kurnit et al. 1984, 1986) and by a locus on 21q11 (G. D. Stewart and D. M. Kurnit, unpublished data). The subcloned fragment, denoted p21-4U, that detects this locus is closely linked to D21S13, the most proximal marker on the 21q linkage map of Tanzi et al. (submitted): no recombinants in 29 informa-

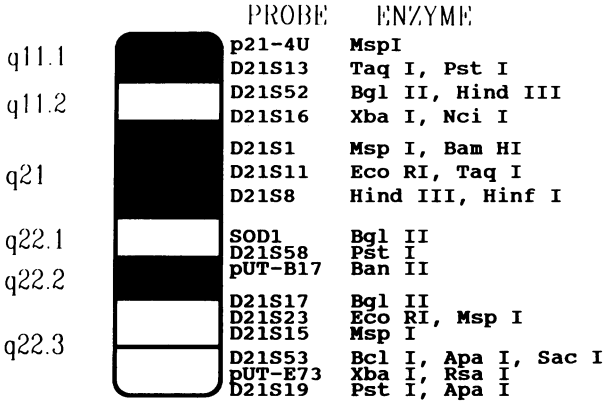


Figure 1 Polymorphic DNA probes on chromosome 21. Each of the 16 DNA probes used in the present study, along with the enzymes used to detect RFLPs, is listed. All the probes have been subregionally mapped on the mapping panel of Van Keuren et al. (1986), and all of the probes except pUT-B17 and pUT-E73 have been ordered on the linkage map of Tanzi et al. (submitted). The order of probes in fig. 1 reflects the above mapping and linkage data.

Table 1

DOWN Program (see Material and Methods) Analysis of Nondisjunction Data in Family 4

Marker	Interpretation	Polymorphism Data		
		Father	Mother	T21
p21-4U	Uninformative	12	12	112
D21S13	Maternal	22	11	112
D21S52	If paternal, meiosis 1	12	11	112
D21S16	If maternal, meiosis 1	22	12	122
D21S1	Uninformative	12	12	112
D21S11	Uninformative	12	12	112
D21S8	Maternal meiosis 2	22	12	112
SOD1	If paternal, meiosis 2	12	11	111
D21S58	If paternal, meiosis 2	12	11	111
pUT-B17	Uninformative	11	11	111
D21S17	If maternal, meiosis 2	11	12	111
D21S23	If paternal, meiosis 2	12	22	222
D21S15	If paternal, meiosis 1	12	22	122
D21S53	Uninformative	11	11	111
pUT-E73	If paternal, meiosis 2	12	11	111
D21S19	Uninformative	12	12	122

NOTE.—Five families with trisomy 21 offspring were studied with 16 polymorphic chromosome 21–specific DNA probes that detect RFLPs. Output data and analysis from the program for family 4 (fig. 2) is given. The marker order reflects the order of probes on 21q from 21cen to 21qter. Probes D21S13 and D21S8 define a maternal error. Once this is known, the meiotic stage is determined on the basis of cytogenetic criteria (meiosis I) and the proximal DNA marker D21S16 (if maternal, then meiosis I). For a maternal error, the more distal markers D21S8 and D21S17 indicate a meiosis-II error. Therefore, a crossover has occurred on one of the nondisjoined chromosomes 21, between D21S16 and D21S8. Presenting the data in this ordered fashion facilitates localization of crossover events.

tive meioses were observed between p21-4U and D21S13. The lack of significant linkage disequilibrium between p21-4U and D21S13 allowed us to use p21-4U and D21S13 to establish an informative haplotype at 21q11, just below the centromere.

Data Analysis

We designed a customized PASCAL program, designated DOWN, that utilizes a Lotus 1-2-3 spreadsheet to analyze the nondisjunctional data in an ordered fashion. DOWN can analyze polymorphism data for as many as 60 probes on multiple three-member families (each consisting of mother, father, and offspring with trisomy 21). As many as three alleles per locus are allowed. One slot is assigned to cytogenetic heteromorphisms on 21p; the remaining slots are allotted to DNA probes on 21q. The probes are ordered on the basis of our knowledge of linkage, subregional mapping, and in situ hybridization data (fig. 1, table 1). For each parent two alleles (one for each chromosome 21) are entered, and for the Down syndrome offspring three alleles (again, one for each chromosome 21) are entered. For each probe, the program then distinguishes among the following possibilities: uninformative; meiosis I (either parent); meiosis II (either parent); maternal (meiotic stage unknown); paternal (meiotic stage unknown); maternal I; maternal II; paternal I; paternal II; if maternal (on the basis of other probes), then meiosis I; if maternal, then meiosis II; if paternal, then meiosis I; if paternal, then meiosis II; inconsistent data (e.g., because of paternity error); and no data available. The operator then chooses whether to print the data for all probes or only for informative probes. The printout (see, e.g., table 1) lists DNA polymorphisms on 21q, listing probes in order traveling down the long arm of chromosome 21 (fig. 1). Presentation of the data in this format permits the operator to scan the data rapidly and determine whether parental origin has been determined consistently. If so, the operator then scans the list; analysis of the first probes (i.e., the cytogenetic heteromorphisms above the centromere and the most proximal probes on 21q just below the centromere) lets the operator decide whether meiotic stage has been determined unambiguously. Finally, the operator scans down the list of probes on 21q.

Discrepancies between a proximal probe and a more distal probe used to infer the stage of meiosis delineate the occurrence of a crossover event between these probes on a chromosome 21 involved in nondisjunction.

Results

To analyze the origin of nondisjunction in five families with trisomy-21 offspring, we used cytogenetic polymorphisms and 16 polymorphic DNA probes spanning the long arm from 21q11 to 21q22.3 (fig. 1). On the basis of cytogenetic criteria, we determined parental origin in two families and determined that the error occurred in meiosis I in either parent in a third family. Our DNA analyses assigned parental origin in four of the five families; taken together, cytogenetic plus molecular analyses determined parental origin in all five families (fig. 2).

In family 1 the cytogenetic markers were uninformative, but analysis of the SOD1 locus indicated a paternal meiosis-II error. Knowledge that the error was paternal made the pericentromeric marker p21-4U informative, since this marker data characterized a paternal error as a meiosis-II event. The simplest explanation for this case is nondisjunction at paternal meiosis II, with no evidence for crossing-over between q11 and the midportion of the long arm.

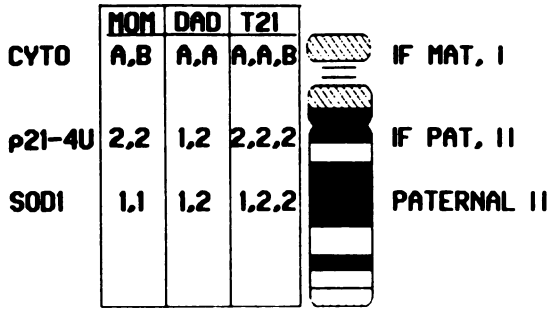
In both family 2 and family 3 the cytogenetic results indicated a maternal meiosis-I error, and in family 2 the maternal origin was confirmed by means of the DNA marker D21S58. There was no evidence in either of these cases for recombination between the two nondisjoined chromosomes, but in family 2 there were no meiotic-stage informative polymorphic loci distal to the D21S1/D21S11 locus, about one-third of the way down the long arm. Future studies of these families will focus on recently acquired distal 21q markers to determine whether a recombination event can be detected.

As documented in figure 3, in family 4 both cytogenetic markers on 21p and the D21S16 DNA marker on the proximal part of the long arm were consistent with a maternal meiosis-I error. However, two more distal 21q DNA markers, D21S8 and D21S17, indicated a maternal meiosis-II error. Crossing-over between two nondisjoined chromosomes can be detected only when the parent of origin is heterozygous for two or more markers. For example, in cases of maternal origin a single (or odd number of) crossover(s) is inferred if (1) the mother is heterozygous for two markers and (2) if the trisomic offspring is homozygous for one marker and heterozygous for the other, having received two maternal chromosomes. Family 4 furnishes an example of such a crossover event: the mother is heterozygous for D21S16 and D21S8, whereas the abortus has both maternal alleles for D21S16 ("maternal meiosis I") but two copies of a single maternal allele for D21S8 ("maternal meiosis II"). Therefore a crossover must have occurred on a nondisjoined chromosome, between the loci identified by D21S16 and D21S8.

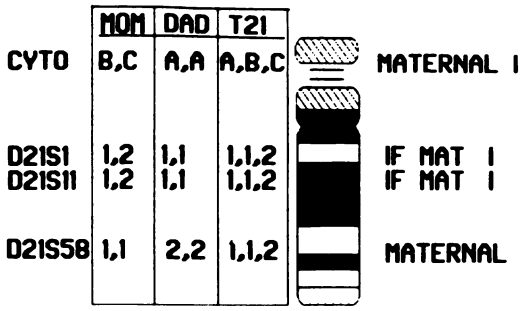
In family 5 D21S17 detected a maternal error. In this family the proximal 21q DNA marker D21S13 indicated a meiosis-II error, whereas the more distal 21q markers D21S58 and D21S19 were consistent with an error at maternal meiosis I. The simplest explanation, which invokes a single crossover for this case, is an error during maternal meiosis II and a crossover between the 21q11 marker D21S13 and the mid–long arm marker D21S58.

Discussion

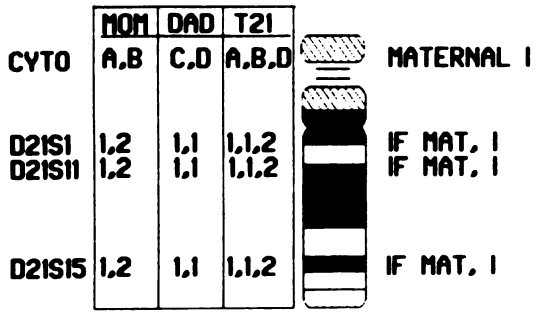
These analyses demonstrate the power of combining molecular and cytogenetic studies of nondisjunction. Using 16 chromosome 21–specific DNA probes, we were able to assign the parental origin of nondisjunction in four of five families; and we used cytogenetic heteromorphisms to define the last case. Further, in all five families, the meiotic stage was determined by using markers tightly linked to the centromere, i.e., chromosomal heteromorphisms on 21p and/or DNA markers on 21q11; this illustrates the importance of establishing an informative DNA-based haplotype in the pericentromeric region of chromosome 21 at 21q11. In all informative cases, the molecular analysis using proximal 21q DNA markers was consistent with the cytogenetic analysis using 21p heteromorphisms. These limited data indicate a low rate of recombination in the pericentromeric area. In turn, such a low rate would agree with the assertion that meiotic crossovers occur rarely in constitutive heterochromatin (John and Miklos 1979; Kurnit 1979). Extension of these studies by using larger numbers of families and polymorphic DNA probes will provide a straightforward test of the relaxed-selection hypothesis, as well as reveal whether reduced levels of recombination are important in nondisjunction. With regard to the latter point, we have already detected crossing-over in one of three cases of maternal meiosis-I origin. Therefore,



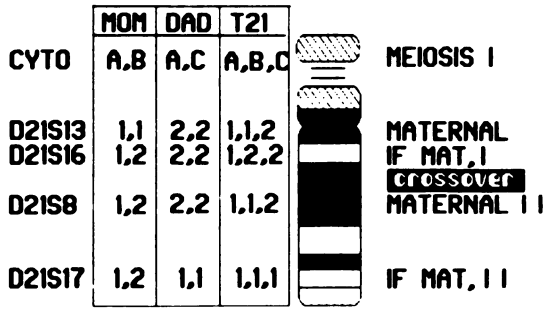
FAMILY 1: PATERNAL II



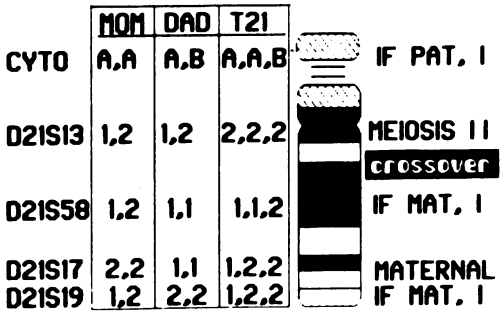
FAMILY 2: MATERNAL I



FAMILY 3: MATERNAL I



FAMILY 4: MATERNAL I



FAMILY 5: MATERNAL II

Figure 2 Nondisjunction in five Down syndrome families as determined by using cytogenetic and DNA polymorphisms. Five families (each comprising mother, father, and Down syndrome offspring) were studied by using cytogenetic heteromorphisms and 16 polymorphic DNA probes spanning chromosome 21. The probes used and sample data output are summarized in table 1. This figure depicts the informative polymorphisms and their relative location on chromosome 21. In families 4 and 5, crossing-over has occurred on a nondisjoined chromosome; in both families, distal probes on 21q are discordant with the stage of meiosis defined by pericentromeric probes.

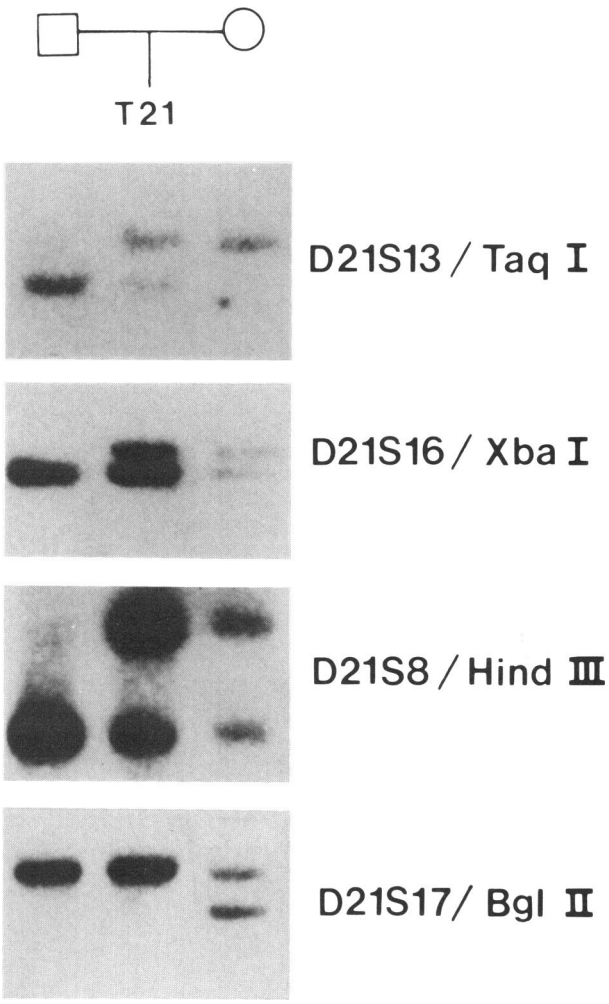


Figure 3 Informative RFLPs in family 4. The Southern hybridizations for the informative probes in this family are shown. In each case, the higher-molecular-weight fragment is listed as allele 1 in table 1 and fig. 2, and the lower-molecular-weight fragment is listed as allele 2.

failure to pair and/or exchange is not the only causative agent in maternal meiosis-I errors to yield trisomy 21.

Future studies of nondisjunction will require objective DNA polymorphic markers on 21q as a supplement to cytogenetic markers on 21p. These DNA markers are required to (1) eliminate bias that might occur by selecting only families with distinguishable cytogenetic polymorphisms and (2) sandwich the centromere between polymorphic markers on 21p and 21q. We note that different questions relating to nondisjunction are to be addressed with DNA polymorphisms; each question requires somewhat different groupings of DNA probes.

1. *The Parental Origin of Nondisjunction*

This question may be answered with any single-copy DNA probe on chromosome 21. Here the goal is to utilize as many markers as feasible. We now have in hand approximately 50 polymorphic DNA markers on chromosome 21 (G. D. Stewart, M. Van Keuren, R. Tanzi, and D. M. Kurnit, unpublished data), so that our efficiency in determining the parental origin of nondisjunction will approach 100% (see Appendix).

2. *The Meiotic Stage during Which Nondisjunction Occurs*

Pericentromeric markers are essential for determining the meiotic stage of origin of nondisjunction. We supplemented cytogenetic studies of 21p heteromorphisms with DNA polymorphisms on 21q just below the centromere. In this manner, we sandwiched the centromere between closely linked markers on the short and long arms, enabling us to rule out recombination between these markers and the centromere. We will expand the p21-4U/D21S13 pericentromeric 21q haplotype by using a recombination-based methodology (Neve et al. 1983; Neve and Kurnit 1983; Seed 1983) that we adapted for chromosome walking in the pericentromeric region of chromosome 21 (Kurnit et al. 1984, 1986). We thereby intend to increase the number of informative probes in 21q11 just below the centromere, making most families informative for at least one locus in the pericentromeric region of 21q. The absence of single-copy probes unique to the short arm of chromosome 21 (Van Keuren et al. 1986; G. D. Stewart, U. Tantravahi, and D. M. Kurnit, unpublished data) requires that cytogenetic heteromophisms be used to mark the short arm of chromosome 21 in nondisjunction studies. Only by simultaneously using flanking pericentromeric markers on 21p (cytogenetic heteromorphisms) and 21q (DNA markers) can the question of meiotic stage be addressed so as to eliminate confounding effects due to crossovers between the centromere and the polymorphic markers.

3. *Recombination during Nondisjunction*

The application of 50 ordered polymorphic probes that span the length of chromosome 21 to a large number of families will permit a rigorous examination of the hypothesis that reduced recombination is associated with nondisjunction (Hassold et al. 1980*b*;

Antonarakis et al. 1986). The effect, if real, would presumably be restricted to meiosis-I errors. Thus, we will test the hypothesis directly by comparing the two classes of errors, i.e., paternal meiosis I versus paternal meiosis II and maternal meiosis I versus maternal meiosis II. If failure to pair or exchange is an important contribution to meiosis I nondisjunction, it should be possible to demonstrate a significant effect by using a moderate number of cases. By using probes that span the length of 21q from 21cen to 21qter, it will be possible to determine whether nondisjunction is associated with altered recombination in specific subregions of chromosome 21 (e.g., in the pericentromeric subregion).

Acknowledgments

This research was supported by National Institutes of Health grants R01 HD20118, R01 HL 37703, and HD21341 and a March of Dimes Clinical Research grant. D.M.K. is an Investigator and G.D.S. is an Associate of Howard Hughes Medical Institute. We thank Yoram Groner for the SOD-1 probe and Uma Tantravahi for access to unpublished probes (pUT-B17, pUT-E73) and data (in situ hybridization studies of probe p21-4U).

Appendix

We anticipate virtually complete ascertainment of the parental origin of nondisjunction on the basis of the following calculations adapted from Stewart (1984). Assume the following: (1) Hardy-Weinberg equilibrium for a two-allele polymorphic system; (2) frequency of the two alleles = p and q, respectively; and (3) 80% of nondisjunction errors that yield trisomy 21 occur in meiosis I and 20% of nondisjunction errors occur in meiosis II.

The parental origin of nondisjunction can be ascertained for both meiosis-I and meiosis-II errors whenever both parents are homozygous for different alleles. The frequency of such matings is $2p^2q^2$. Further, the parental origin of nondisjunction can be ascertained for meiosis-II errors when the error occurring in a heterozygous parent results in duplication of the allele not found in the other (homozygous) parent. (This methodology works for meiosis-I errors—but not for meiosis-II errors—when a crossover has occurred between the centromere and the probe locus on the relevant chromosome; this case, which would be favorable to our calculation, is neglected in the equations below, in which we use .2 [see above] as the proportion of useful [i.e., meiosis-II] errors.) The probability, per mating, of obtaining an informative meiosis II error is $.2[(pq)(p^2 + q^2)]$. To sum, the probability P that a given probe can determine the parental origin is $P = 2p^2q^2 + .2[(pq)(p^2 + q^2)]$. When a typical minor-allele frequency of .2 ($q = .2$) is used, $P = .073$/probe. Since we have as many as 50 probes to utilize for each family, the probability that parental origin cannot be determined after using 50 such DNA polymorphisms is low, i.e., $(1 - .073)^{50} = \sim 1/50$. Further, since cytogenetic heteromorphisms (which determine parental origin in $\geq 50\%$ of cases) will also be used, we anticipate that we will achieve virtually complete ascertainment of parental origin. These expectations have been confirmed in our pilot studies, in which we successfully determined parental origin in all five families examined with both cytogenetic heteromorphisms and 16 polymorphic DNA probes (the expected rate of failure per experiment with DNA polymorphisms alone when using this order-of-magnitude calculation would be $[1 - 0.073]^{16} = \sim 1/3$, in reasonable agreement with our 1/5 failure rate when using 16 polymorphic DNA probes; see fig. 2). In future studies, by using 50 probes in this manner, or probes with higher minor-allele frequencies, or probes that detect multiple-allele polymorphisms, we will avoid biases that underestimate meiosis-I errors because of incomplete ascertainment.

References

Antonarakis, S. E., A. Chakravarti, A. C. Warren, S. A. Slaugenhaupt, C. Wong, S. L. Halloran, and C. Metaxotou. 1986. Reduced recombination rate on chromosomes 21 that have undergone nondisjunction. Cold Spring Harbor Symp. Quant. Biol. 51:185–190.

Aymé, S., C. Baccichetti, F. D. Bricarelli, B. Dallapiccola, D. Lungarotti, M. Mikkelsen, and M. Nevin. 1986. Factors involved in chromosomal nondisjunction: a European collaborative study supported by the E.E.C. 7th International Congress of Human Genetics. Abstracts, Pt. I, C IV. 19.

Aymé, S., and A. Lippman-Hand. 1982. Maternal-age effect in aneuploidy: does altered embryonic selection play a role? Am. J. Hum. Genet. 39:558–565.

Biddle, F., B. Eates, and L. Oland. 1986. Strain difference in survival of XO embryos in the mouse. Am. J. Hum. Genet. 39:A104.

Bott, C. E., B. S. Sekhon, and H. A. Lubs. 1975. Unexpected high frequency of paternal origin of trisomy 21. Am. J. Hum. Genet. 27:20A.

Carothers, A. 1987. Down syndrome and maternal age: the effect of erroneous assignment of parental origin. Am. J. Hum. Genet. **40**:147–150.

Davies, K. E., K. Harper, D. Bonthron, R. Krumlauf, A. Polkey, M. E. Pembery, and R. Williamson. 1984. Use of a chromosome 21 cloned DNA probe for the analysis of nondisjunction in Down's syndrome. Hum. Genet. **66**:54–56.

Degau, J. H., J. H. van de Sande, and C. C. Lin. 1978. $(CMA)_2S$: a double intercalating analogue of quinacrine for chromosome banding. Am. J. Hum. Genet. **30**:78A.

del Mazo, J., A. M. Castillo, and J. A. Abrisqueta. 1982. Trisomy 21: origin of nondisjunction. Hum. Genet. **62**:316–320.

Feinberg, A. P., and B. Vogelstein. 1983. A technique for radiolabeling restriction fragments to high specific activity. Anal. Biochem. **132**:6–13.

Hansson, A., and M. Mikkelsen. 1978. The origin of the extra chromosome 21 in Down syndrome: studies of fluorescent variants and satellite association in 26 informative families. Cytogenet. Cell Genet. **20**:194–203.

Hassold, T., N. Chen, J. Funkhouser, T. Jooss, B. Manuel, J. Matsuura, and A. Matsuyama. 1980*a*. A cytogenetic study of 1,000 spontaneous abortions. Ann. Hum. Genet. **44**:151–178.

Hassold, T., D. Chiu, and J. Yamane. 1984. Parental origin of autosomal trisomies. Ann. Hum. Genet. **48**:129–144.

Hassold, T., P. A. Jacobs, J. Kline, Z. Stein, and D. Warburton. 1980*b*. Effect of maternal age on autosomal trisomies. Ann. Hum. Genet. **44**:19–36.

Hassold, T., P. A. Jacobs, and D. Pettay. 1987. Analysis of nucleolar organizing regions in parents of trisomic spontaneous abortions. Hum. Genet. **76**:381–384.

Henderson, S. A., and R. G. Edwards. 1968. Chiasma frequency and maternal age in mammals. Nature **218**:22–28.

Hook, E. B. 1983. Down syndrome rates and relaxed selection at older maternal ages. Am. J. Hum. Genet. **35**: 1307–1313.

Houghton, J. A. 1981. The study of chromosome nondisjunction in man. Ir. J. Med. Sci. **150**:357–366.

Jacobs, P. A., and M. Mayer. 1981. The origin of human trisomy: a study of heteromorphisms and satellite associations. Ann. Hum. Genet. **45**:49–57.

Jagiello, G., and J. S. Fang. 1979. Analyses of diplotene chiasma frequencies in mouse oocytes and spermatocytes in relation to aging and sexual dimorphism. Cytogenet. Cell Genet. **23**:53–60.

John, B., and G. L. G. Miklos. 1979. Functional aspects of satellite DNA and heterochromatin. Int. Rev. Cytol. **58**:1–114.

Juberg, R. C., and P. N. Mowrey. 1983. Origin of nondisjunction in trisomy 21 syndrome: all studies compiled, parental age analysis, and external comparisons. Am. J. Med. Genet. **16**:111–116.

Kunkel, L. M., K. D. Smith, S. H. Boyer, D. S. Borgaonkar, S. S. Wachtel, O. J. Miller, W. R. Breg, H. W. Jones, and J. M. Rary. 1977. Analysis of human Y-chromosome specific reiterated DNA in chromosome variants. Proc. Natl. Acad. Sci. USA **74**:1249–1254.

Kurnit, D. M. 1979. Satellite DNA and heterochromatin variants: the case for unequal mitotic crossing over. Hum. Genet. **47**:169–186.

Kurnit, D. M., R. L. Neve, C. C. Morton, G. A. P. Bruns, N. S. F. Ma, D. R. Cox, and H. P. Klinger. 1984. Recent evolution of DNA sequence homology in the pericentromeric regions of human acrocentric chromosomes. Cytogenet. Cell Genet. **38**:99–105.

Kurnit, D. M., S. Roy, G. D. Stewart, J. Schwedock, R. L. Neve, G. A. P. Bruns, M. L. Van Keuren, and D. Patterson. 1986. The 724 family of DNA sequences is interspersed about the pericentromeric regions of human acrocentric chromosomes. Cytogenet. Cell Genet. **43**:109–116.

Laurie, D. A., and M. A. Hultén. 1985. Further studies on bivalent chiasma frequency in human males with normal karyotypes. Ann. Hum. Genet. **49**:189–201.

Lieman-Hurwitz, J., N. Dafni, V. Lavie, and Y. Groner. 1982. Human cytoplasmic superoxide dismutase cDNA clone: a probe for studying the molecular biology of Down syndrome. Proc. Natl. Acad. Sci. USA **79**:2808–2811.

Luthardt, F. W. 1977. Cytogenetic analyses of human oocytes. Am. J. Hum. Genet. **29**:71A.

Luthardt, F. W., C. G. Palmer, and P. -L. Yu. 1973. Chiasma and univalent frequencies in aging female mice. Cytogenet. Cell Genet. **12**:68–79.

Magenis, R. E., K. M. Overton, J. Chamberlin, T. Brady, and E. Lovrien. 1977. Prenatal origin of the extra chromosome in Down's syndrome. Hum. Genet. **37**:7–16.

Mattei, J. F., S. Aymé, M. G. Mattei, and F. Giraud. 1980. Maternal age and origin of nondisjunction in trisomy 21. J. Med. Genet. **17**:368–372.

Mikkelsen, M., P. Hanne, G. Jorgen, and L. Aksel. 1980. Nondisjunction in trisomy 21: study of chromosomal heteromorphisms in 110 families. Ann. Hum. Genet. **44**:17–28.

Neve, R. L., G. A. P. Bruns, T. P. Dryja, and D. M. Kurnit. 1983. Retrieval of human DNA from rodent-human libraries by a recombination process. Gene **23**:343–354.

Neve, R. L., and D. M. Kurnit. 1983. Comparison of sequence repetitiveness of human cDNA and genomic DNA using the recombination miniplasmid, piVX. Gene **23**:355–367.

Neve, R. L., G. D. Stewart, P. Newcomb, M. L. Van Keuren, D. Patterson, H. A. Drabkin, and D. M. Kurnit. 1986. Human chromosome 21–encoded cDNA clones. Gene **49**:361–369.

Penrose, L. S. 1933. The relative effect of paternal age and maternal age in mongolism. J. Genet. **27**:219–224.

Polani, P. E., and G. M. Jagiello. 1976. Chiasmata, meiotic univalents and age in relationship to aneuploid imbalance in mice. Cytogenet. Cell Genet. **16**:505–529.

Roberts, D. F., and M. H. Callow. 1980. Origin of the additional chromosome in Down's syndrome. J. Med. Genet. **39**:68–78.

Seed, B. 1983. Purification of genomic sequences from bacteriophage libraries by recombination and selection in vivo. Nucleic Acids Res. **11**:2427–2445.

Speed, R. M. 1977. The effects of aging on the meitoic chromosomes of male and female mice. Chromosoma **64**:241–254.

Speed, R. M., and A. C. Chandley. 1983. Meiosis in the foetal mouse ovary. II. Oocyte development and age-related aneuploidy: does a production line exist? Chromosoma **88**:184–189.

Stein, Z., W. Stein, and M. Susser. 1986. Attrition of trisomies as a maternal screening device. Lancet **1**:944–946.

Stewart, G. D. 1984. The isolation and characterisation of cloned DNA from a chromosome 21 enriched DNA library. Ph.D. thesis, University of Glasgow.

Stewart, G. D., P. Harris, J. Galt, and M. A. Ferguson-Smith. 1985. Cloned DNA probes regionally mapped to human chromosome 21 and their use in determining the origin of nondisjunction. Nucleic Acids Res. **3**:4125–4132.

Sugawara S., and K. Mikamo. 1983. Absence of correlation between univalent formation and meiotic nondisjunction in the aged female Chinese hamster. Cytogenet. Cell Genet. **35**:34–40.

Tanzi, R. E., J. L. Haines, G. D. Stewart, P. C. Watkins, K. T. Gibbons, A. G. Faryniarz, M. R. Wallace, R. Hallewell, E. Yount, N. S. Wexler, P. M. Conneally, and J. F. Gusella. Genetic linkage map for the long arm of chromosome 21 (submitted).

Van Keuren, M. L., P. C. Watkins, H. A. Drabkin, E. W. Jabs, J. F. Gusella, and D. Patterson. 1986. Regional localization of DNA sequences on chromosome 21 using somatic cell hybrids. Am. J. Hum. Genet. **38**:793–804.

Vekemans, M., and D. Trasler. 1985. Maternal genes control the extent of in utero selection against trismic embryos in the mouse. Am. J. Hum. Genet. **37**:A128.

Wagenbichler, P., W. Killian, A. Rett, and W. Schnedl. 1976. Origin of the extra chromosome no. 21 in Down's syndrome. Hum. Genet. **32**:13–16.

Watkins, P. C., R. E. Tanzi, K. T. Gibbons, J. V. Tricoli, G. Landes, R. Eddy, T. B. Shows, and J. F. Gusella. 1986. Isolation of polymorphic DNA segments from human chromosome 21. Nucleic Acids Res. **13**:6075–6088.