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Trisomy 21: Association between Reduced Recombination and Nondisjunction

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Summary

To assess the association between recombination and nondisjunction of chromosome 21, we analyzed cytogenetic and DNA markers in 104 trisomy 21 individuals and their parents. Our DNA marker studies of parental origin were informative in 100 cases, with the overwhelming majority (94) being maternal in origin. This value is significantly higher than the 75%–80% maternal nondisjunction rate typically observed in cytogenetic studies of trisomy 21 and illustrates the increased accuracy of the molecular approach. Using the maternally derived cases and probing at 19 polymorphic sites on chromosome 21, we created a genetic map that spans most of the long arm of chromosome 21. The map was significantly shorter than the normal female linkage map, indicating that absence of pairing and/or recombination contributes to nondisjunction in a substantial proportion of cases of trisomy 21.

Introduction

De Grouchy (1970) and Juberg and Jones (1970) were the first to use chromosome heteromorphism analysis to study the parental origin of trisomy 21. Since then, over 1,000 Down syndrome individuals and parents have been similarly studied using Q- and/or other banding techniques, and the results have been summarized in several reviews (e.g., Juberg and Mowrey 1983; Hassold and Jacobs 1984; Bricarelli et al. 1989). Most studies have reported a high frequency of maternal meiosis I errors, and it is virtually certain that nondisjunction at this stage is the most common source of trisomy 21. However, a substantial proportion of cases have been attributed to paternal nondisjunction. For example, in summarizing their own work and that of others, Bricarelli et al. (1989) reported a paternal nondisjunction rate of 24% (121 of 497 cases). This high frequency is somewhat surprising, given the strong association between increasing maternal age and the incidence of trisomy 21 (Hook 1981), but it is generally accepted that paternal nondisjunction is responsible for 20%–25% of trisomy 21.

The identification of DNA polymorphisms provides an alternate approach to studying the parental origin of trisomy 21 and, thereby, a means for assessing the validity of the cytogenetic observations. Furthermore, the availability of multiple polymorphic loci on 21q makes it possible to address questions which cannot be approached using only cytogenetic methodology. One of the most interesting of these is the possibility that nondisjunction is associated with abnormally low or high levels of recombination. Over 20 years ago, Henderson and Edwards (1968) proposed that maternal age–related trisomy might be due to decreased chiasma frequency in aging oocytes. More recently, Warren et al. (1987) provided the first direct evidence of an association between reduced recombination and human trisomy. In an analysis of DNA markers in 34

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Down syndrome families, they observed a significant reduction in crossing-over on the chromosomes 21 that had undergone nondisjunction. However, the limited number of available probes prevented Warren et al. (1987) from analyzing the terminal region of 21q, and they did not correlate their observations on recombination with information on maternal age.

Other laboratories, including our own, also have studied recombination in nondisjoined chromosomes 21 but have failed to confirm the observations of Warren et al. (1987) (Roulston et al. 1989; Hamers et al. 1990; Takaesu et al. 1990). However, our results were based on a relatively small number of Down syndrome families; therefore, we have extended our study of cytogenetic and DNA polymorphisms in trisomy 21 conceptuses and their parents, in order to (1) determine the parental origin of the additional chromosome, (2) evaluate the possible role of aberrant recombination in the genesis of trisomy 21, and (3) correlate the observations on parental origin and recombination with information on maternal age. In the present report, we summarize our observations on a series of 104 Down syndrome families. Our results indicate a paternal nondisjunction rate of 6%, approximately the same value as that recently reported by Antonarakis et al. (1991) and much lower than that suggested by the cytogenetic studies. In studies of recombination, we observed a significant reduction in exchange frequency in meioses leading to trisomy 21, suggesting an important role for pairing/recombination failure or reduced recombination in the etiology of trisomy 21.

Material and Methods

Study Population

The present study population consists of 104 trisomy 21 conceptions and their parents. In all instances, cytogenetic studies were consistent with nonmosaic trisomy 21. Seventy-nine of the cases were live-born individuals with clinical features of Down syndrome. Eighteen cases were therapeutic abortions, most (15 cases) of which were studied prenatally because of advanced maternal age. Seven cases were trisomic fetuses ascertained in a cytogenetic survey of spontaneous abortions.

Preliminary cytogenetic and molecular analyses on a subset of these cases have been presented elsewhere (Takaesu et al. 1990). Cases common to the two studies are indicated by identification numbers with the prefix "D" in the previous report.

Cytogenetic Methodology

Routine cytogenetic studies were done on all cases and parents, using standard Q- or G-banding procedures. For determinations of parent and meiotic stage of origin of nondisjunction, chromosome heteromorphisms of the parents and trisomic fetuses or live borns were compared using either Q-banding or, in some cases, Q-banding and NOR staining. All cases were examined by at least two independent observers, either directly under the microscope (Atlanta and Salisbury centers), using photographs of Q-banded preparations (Hamilton center), or using both direct microscopy and photographic analysis (Copenhagen center). In the event of a disagreement among observers, the final decision taken was the most conservative one compatible with both sets of observations.

DNA Studies

DNA was prepared from either peripheral blood samples or frozen fetal tissue as described elsewhere (Hassold et al. 1985). For subsequent Southern blotting studies, DNA samples were digested with the appropriate restriction enzymes under conditions specified by the supplier (Bethesda Research Labs), size fractionated on 0.6%–1.5% agarose gels, and transferred to Zetabind membranes (AMF-Cuno) by using the method of Southern (1975). Membranes were hybridized under standard conditions with ^{32}P-radiolabeled probes (Feinberg and Vogelstein 1983). Probes detecting 19 polymorphisms at 15 chromosome 21–specific DNA loci—D21S1 (pPW228C), D21S13E (pGSM21), D21S15 (pGSE8), D21S16 (pGSE9), D21S17 (pGSH8), D21S19 (pGSB3), D21S55 (pPW518), D21S58 (pPW524–5P), D21S110 (p21–44), D21S112 (CRI-L427), D21S113 (pMCT15), SOD1 (pSOD), ETS2 (pHO33), CD18 (3.1.1), and COL6A1 (pML18)—were used in the study. Information on the polymorphisms detected by these probes and on their chromosomal location is described elsewhere (Kidd et al. 1989).

Genetic Linkage Studies

Trisomy 21 families.—Centromere-gene mapping methods were used to estimate the amount of recombination along chromosomes 21 involved in maternal nondisjunction. For this type of analysis, the estimation of recombination depends on identifying the parental origin of the nondisjunction and on identifying genetic markers for which the parent of origin is heterozygous. The markers are then evaluated in the trisomic

offspring to determine whether heterozygosity is maintained (nonreduction) or reduced to homozygosity (reduction). Chakravarti and Slaugenhaupt (1987), Chakravarti et al. (1989), and Morton et al. (1990) have recently discussed the application of this approach to the study of autosomal trisomies and of maternally derived sex chromosome trisomies, respectively. The probability that the disomic gamete was heterozygous (y), also called the probability of nonreduction (Morton and MacLean 1984), was used as a linkage parameter and was estimated using maximum likelihood approaches. Genetic distances between two genetic markers were estimated using the more proximal locus as a "pseudocentromere" to establish the conditions under which the probability of nonreduction was evaluated (Morton et al. 1990). As no centromeric marker was available, centromere-gene distances could not be estimated.

Fifteen chromosome 21 loci were studied in each of the 77 maternally derived trisomy 21 conceptuses and their parents. For each locus at which the mother was heterozygous, the trisomy 21 offspring was categorized as follows: N = nonreduced or retained heterozygosity (e.g., FA:11 × MO:12; DS:112), R = reduced or loss of heterozygosity (e.g., FA:11 × MO:12; DS:111), PI = intercross with partial information on heterozygosity (e.g., FA:12 × MO:12; DS:112), or U = uninformative (table 4). Information at the two most proximal loci D21S16 and D21S13E and at D21S112, D21S113, and CD18 were combined, as no recombinants were observed within each group.

The maximum likelihood estimates of the probability of nonreduction (y) between all pairs of markers was obtained using the methods outlined by Shahar and Morton (1986) and implemented in the computer program TETRAD. For each marker pair, the estimated probability of nonreduction was based on the number of trisomy 21 cases for which (1) both markers were nonreduced (N→N), (2) both were reduced (R→R), or (3) one was nonreduced and the other reduced (N→R). The first two categories are consistent with no recombination, while the third indicates that recombination has occurred. From these data, recombination fractions and lod scores were derived from the estimated probability of nonreduction by assuming at most two chiasmata within any interval, and these are shown in table 5. Lod scores were based on the likelihood ratio $\log_{10}[L(\hat{y})/L(y=2/3)]$, where $y = 2/3$ indicates no linkage (Mather 1938).

Chromosomally normal individuals.—Female two-point recombination fractions and lod scores for chromosome 21–specific markers were obtained from published linkage data which are maintained in the LOD-SOURCE data base by one of us (References and linkage data are available, on request, from B.J.B.K.). The summary linkage data are shown in table 5.

Comparison of genetic maps based on nondisjoined chromosomes 21 and from normal chromosomes 21.—The order of genetic markers used in the present study has been well established by both physical and genetic mapping studies (e.g., Tanzi et al. 1988; Warren et al. 1989; Gardiner et al. 1990; Burmeister et al. 1991) and is given as cen–(D21S16, D21S13E)–D21S110–D21S1–SOD1–D21S58–D21S17–D21S55–ETS2–D21S15–D21S19–(D21S112, D21S113, CD18)–COL6A1–qter. On the basis of this order, the interval distances between adjacent markers were estimated from the recombination fractions and lod scores for all pairwise combinations of markers, by using the computer program MAP (Morton and Andrews 1989). By this procedure, the total lod score over all pairs of markers was maximized to give the best estimates of interval distances. Map intervals were estimated in centimorgans (cM), under the assumption that the interference level was $P = .35$, by using the Rao map function (Rao et al. 1977).

The relationship between map intervals of the two genetic maps was defined in two ways, to test the different hypotheses. First, to determine whether there was a difference in the overall amount of recombination between the genetic maps derived from normal and trisomy-generating meioses, we assumed a constant map-distance ratio, k, between the two maps. Thus, each map interval, i, was estimated simultaneously as $w_i^{tri} = kw_i^{nor}$, where w_i^{tri} and w_i^{nor} equal the map distance (in cMs) for the ith interval of the trisomic and normal female maps, respectively. If there were no association between recombination and nondisjunction, the map distance ratio would equal 1. Thus, to test this association, the likelihood of two maps estimated assuming $k = 1$ ($L_{k=1}$) was compared with the likelihood obtained when k was estimated ($L_{\hat{k}}$). Significance was tested as $\chi_1^2 = 21nL_{\hat{k}} - 21nL_{k=1}$. When a significant result was obtained, a second test was performed to determine whether the distribution of chiasmata along the chromosome arm differed between maps. If the distribution was similar, the map-distance ratio would remain constant over each interval along the chromosome arm. However, if there was more or less recombination in specific regions of the trisomic map compared with the normal map, estimation of the map-distance ratio within each

interval would significantly increase the likelihood. Thus, maps were generated, estimating each map interval independently, as $w_i^{tri} = k_i w_i^{nor}$, where k_i is estimated for each map interval. The likelihood obtained was compared with the likelihood of the two maps by assuming a constant ratio, k, as described above. Significance was tested as above, with df = $2n - (n+1)$, where n is the number of intervals being estimated.

Comparison of cases with inferred meiosis I and II errors. — A simple comparison of cases was used to determine whether there was a correlation between meiotic stage of origin and either the amount of crossing-over or maternal age. As we wanted to ensure that a reasonable amount of information was available to detect a recombinant event, we included only those cases which were informative for recombination at one or more loci in each of the following three intervals that spanned the chromosome 21 long arm: (1) D21S16, D21S13E, D21S110, and D21S1; (2) SOD1, D21S58, D21S17, D21S55, ETS2, D21S15, and D21S19; and (3) D21S112, D21S113, CD18, and COL6A1. As no centromeric marker was available, we used information from the first informative proximal marker to infer the meiotic stage of origin of the nondisjunctional error. Thus, a meiosis I error was inferred if nonreduction were observed at a marker in the first of the three intervals, and a meiosis II error was inferred if reduction were observed. These assignments would be in error if recombination occurred between the centromere and the proximal marker (see Discussion).

Results

Cytogenetic Studies of Parental Origin

Chromosome preparations from the father, mother, and trisomic offspring were available for 98 of the 104 families. The results of the cytogenetic analyses of these families are summarized in table 1. We made a decision on parental origin in 25 cases, with 24 being attributed to maternal nondisjunction and one being attributed to paternal nondisjunction. However, in subsequent DNA marker studies, we identified two cases in which the molecular and cytogenetic assignments of parental origin differed. In one instance a trisomy scored as paternal on cytogenetic analysis was scored as maternal on DNA analysis, and in the other case a trisomy scored as maternal cytogenetically was paternal on DNA analysis. In each case the molecular assignments were confirmed at several loci, with the conclusion being based on results at six loci in one instance and on results at four loci in the other. Thus, it seems likely that the molecular assignments were correct and that two (8%) of the 25 cytogenetic determinations were in error. Because of this relatively high error rate, our subsequent analyses of parental origin were based solely on the DNA marker studies. For this same reason, we made no attempt to correlate the cytogenetic analyses of meiotic stage of origin with the results of the DNA studies.

Molecular Studies of Parental Origin

The results of the DNA marker studies of parental origin are summarized in table 2 (see example in fig. 1). We were able to specify the parent of origin of trisomy in 97 of the 104 cases, with 91 being maternally derived and six being paternally derived. In three other families, in which only the proband and mother were available for study, our observations were compatible with maternal nondisjunction with each of 13–18 polymorphisms studied, including three to five VNTRs. It is probable that these trisomies were also maternally derived, and, if we consider them as such, the overall number of cases of maternal and paternal origin become 94 and six, respectively.

We were unable to determine the parental origin in the remaining four cases. In two cases, only two or three loci have yet been studied, and these are likely

Table 1

Summary of Cytogenetic Studies of Parental Origin, and Comparison of Results with Those of DNA Marker Studies

Parental Origin of Trisomy	Total No. of Cases	No. Concordant with DNA Analysis	No. Discordant with DNA Analysis
Paternal	1	0	1
Maternal	24	23	1
Unknown	73	. . .	. . .

Table 2

Parental Origin of Trisomy 21, by Ascertainment Category of Proband

Parental Origin of Trisomy	Total No. of Cases	No. in Ascertainment Category			
		Spontaneous Abortion	Therapeutic Abortion—Maternal Age	Therapeutic Abortion—Other	Live Born
Paternal	6	0	0	1	5
Maternal	94[a]	6	14	2	72
Unknown	4	1	0	1	2

[a] Includes three cases (one therapeutic abortion studied for advanced maternal age and two live borns) in which DNA was available only from the trisomy and mother and in which results were consistent with maternal origin at all loci studied.

to become informative when more loci are evaluated. However, the other two cases were uninformative with 19 and 17 polymorphisms, respectively. These were the only families in which we were unable to determine the parental origin of trisomy, despite extensive studies and the availability of DNA samples from both parents and the trisomic offspring.

Table 3 shows the distribution and mean maternal ages for cases of maternal and paternal origin. There were no obvious differences between the maternally and paternally derived cases, but the number of cases of paternal origin is small.

Recombination Studies

We evaluated recombination between the two nondisjoined chromosomes for 82 cases in which the parental origin of nondisjunction was known and in which DNA samples were available from both parents. Five of the paternally derived trisomies were evaluated; in three cases we identified a single exchange, and in the other two cases all informative loci were reduced to homozygosity (table 4). As there were only five cases, further analyses were not performed.

Seventy-seven of the maternally derived trisomies were studied, and the information on these cases is also presented in table 4. We identified a single exchange in 20 cases and two exchanges in five cases. No trisomy with more than two crossovers was identified. Among the remaining 52 cases in which we were unable to detect recombination, four cases were reduced to homozygosity at all informative loci, and 48 cases were heterozygous at all informative loci. However, among the latter group, three cases were informative at only one locus, eliminating the possibility of detecting recombination, and another seven cases were informative at only two loci.

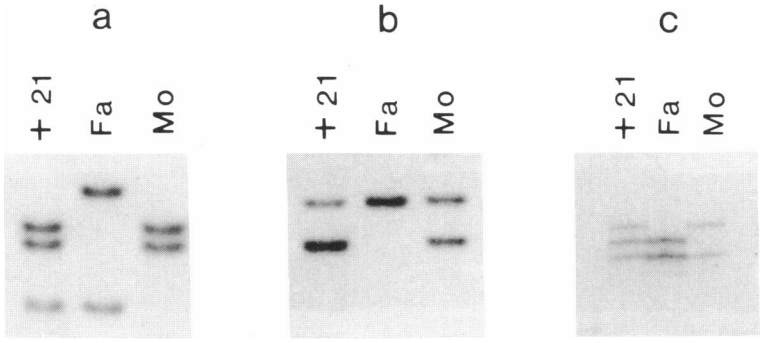

Figure 1 Examples of DNA marker studies in trisomy 21 individuals and their parents. *a,* Trisomy of maternal origin, since trisomic individual has inherited two maternal alleles and one paternal allele (locus/probe/enzyme = D21S112/CRI-L427/*Rsa*I). *b,* Trisomy of maternal origin, since trisomic individual has inherited two copies of one maternal allele and one paternal allele (locus/probe/enzyme = D21S13/pGMS21/*Taq*I). *c,* Trisomy of unknown parental origin, but with meiotic, and not mitotic, origin, since trisomic individual has inherited three different parental alleles (locus = HMG14-GT1; dinucleotide repeat polymorphism detected by using PCR as described by Petersen et al. [1990]; this marker was studied only in subset of present study population and, therefore, was not included in overall analysis).

Table 3

Information on Maternal Age, by Parent of Origin of Trisomy

Parental Origin of Trisomy	Total No. of Cases	No. in Maternal Age Group					Mean ± SD Maternal Age (years)
		≤24 years	25–29 years	30–34 years	35–39 years	≥40 years	
Paternal	6	0	3	1	2	0	31.2 ± 4.5
Maternal	80[a]	14	26	23	13	4	30.1 ± 5.4
Unknown	3[b]	0	2	1	0	0	28.3 ± 2.5

[a] Excludes 14 cases ascertained for advanced maternal age.
[b] Excludes one case ascertained for advanced maternal age.

We used the information on recombination from these 77 maternal cases to generate a trisomy-based genetic map and compared this with the female genetic map generated from conventional linkage studies (table 5). We found a significant difference between maps: the genetic map associated with maternal nondisjunction was only one-third the length of the conventional map ($k \pm$ standard error $= 0.33 \pm 0.06$, $\chi^2_1 = 46.92$, $P < .001$). To determine whether the distribution of chiasmata differed between maps, we estimated each map separately and compared it with the maps by assuming a constant map-distance ratio. We found no evidence that recombination was reduced in specific regions of the nondisjoined chromosomes ($\chi^2_{10} = 12.42$, $P > .05$), although the data are sparse.

Given this highly significant reduction in recombination in a proportion of the nondisjoined chromosomes, we examined the relationship between maternal age and recombination in the 77 maternal cases. For this analysis, we compared the genetic map based on the 36 cases involving mothers ≤30 years of age with that based on the 40 cases involving mothers >30 years of age (no age was available on one of the mothers). There was no evidence for a difference between maps (data not shown). However, the validity of this approach is questionable, since, in the absence of a centromeric marker, maternal meiosis I and II errors cannot be unequivocally discriminated.

For this reason, we separated maternal trisomies on the basis of the most proximal marker (see Material and Methods), equating cases with a nonreduced proximal marker to meiosis I nondisjunction and those with a reduced proximal marker to meiosis II nondisjunction. On the basis of this definition, we found a significant difference in mean maternal age ($P = .04$), as mothers with meiosis I errors were approximately 4 years older than mothers with meiosis II errors (table 6).

We then examined the number of detectable crossovers among cases with inferred meiosis I and II errors and found that there were significantly fewer crossovers among those due to meiosis I errors compared with those due to meiosis II errors ($P = .002$; table 7). Thus these data suggest that both the maternal age effect and reduced recombination are restricted to meiosis I errors.

To determine whether the increase in maternal age was correlated with decreased recombination in these inferred meiosis I errors, we examined the age distribution and mean age of mothers with no, one, or two detectable crossovers (table 8). We did not find a significant difference, but the number of cases in each of the categories is limited.

Discussion

The Parental Origin of Trisomy 21

The results of the present study indicate that paternal nondisjunction is responsible for approximately 6% of cases of trisomy 21. This is a somewhat surprising observation, since cytogenetic studies have suggested a paternal error rate of 20%–25% (e.g., see Bricarelli et al. 1989). There are at least three possible explanations for this discrepancy. First, it may be that the method of ascertainment of cases differs between the previous cytogenetic studies and the present one, with our study being biased toward ascertaining maternally derived trisomies. Most cytogenetic studies have been based on live-born individuals, while a rela-

Table 4

Analysis of Recombination in Five Cases of Paternally Derived Trisomy 21 and in 77 Cases of Maternally Derived Trisomy 21

ID No. (maternal age in years)	Status at Locus (cen→qter)[a]											
	D21S16/ D21S13E	D21S110	D21S1/ D21S11	SOD1	D21S58	D21S17	D21S55	ETS2	D21S15	D21S19	D21S112/ D21S113/CD18	COL6A1
Paternal trisomy 21:												
D30 (27)	U	U	R	U	R	U	PI	R	PI	U	N	N
D75 (27)	R	U	R	U	U	R	R	U	U	R	U	U
D92 (36)	U	R	U	U	U	PI	N	N	PI	U	N	N
D136 (37)	R	R	U	N	U	NT	N	N	PI	U	U	U
D156 (28)	R	R	R	U	R	R	R	U	R	U	R	U
Maternal trisomy 21:												
D1 (23)	N	U	N	N	PI	U	N	U	U	N	N	N
D3 (35)	U	N	N	U	NT	NT	NT	U	U	NT	N	U
D4 (37)	U	U	N	N	U	U	N	R	PI	U	R	U
D5 (32)	U	N	U	U	N	NT	NT	U	PI	U	N	R
D7 (22)	U	U	N	U	U	U	U	U	N	U	N	U
D8 (31)	U	U	N	U	PI	U	U	R	R	R	R	R
D14 (30)	R	U	R	U	U	N	NT	N	U	U	R	R
D15 (21)	U	U	U	U	U	N	NT	U	U	U	PI	R
D18 (20)	U	U	PI	U	PI	N	NT	U	PI	U	R	R
D21 (26)	R	N	N	N	U	U	NT	N	U	N	N	N
D22 (33)	N	PI	N	U	N	U	NT	N	U	N	N	U
D31 (36)	U	N	U	U	N	U	N	U	PI	R	N	N
D32 (35)	R	R	R	U	R	U	R	U	PI	R	R	R
D33 (38)	PI	U	N	U	PI	PI	N	PI	U	N	U	N
D35 (29)	PI	U	N	U	PI	N	U	U	PI	U	N	U
D36 (40)	NT	NT	NT	NT	U	NT	NT	U	NT	N	N	NT
D37 (24)	PI	U	R	U	U	N	N	U	PI	U	N	N
D38 (25)	NT	NT	NT	NT	PI	U	NT	U	NT	U	N	NT
D39 (42)	NT	NT	NT	NT	PI	U	NT	U	NT	U	N	NT
D40 (32)	R	U	R	U	PI	R	R	R	PI	N	N	N
D42 (26)	U	N	U	U	U	U	NT	U	N	U	PI	N
D45 (48)	U	PI	PI	U	N	N	NT	U	N	U	N	N
D53 (28)	PI	U	PI	N	PI	U	PI	U	PI	U	N	NT
D55 (31)	PI	U	R	U	U	U	PI	N	U	U	N	N
D56 (29)	N	N	PI	U	U	U	N	N	U	N	N	PI
D57 (29)	PI	U	U	U	N	N	N	PI	PI	U	R	N
D58 (31)	N	U	U	U	PI	U	R	U	R	U	R	R
D59 (31)	U	U	N	U	U	U	N	PI	PI	U	R	U
D60 (33)	N	U	N	U	N	N	U	N	U	U	N	N
D61 (37)	U	U	U	U	PI	U	U	PI	N	U	N	N
D62 (23)	R	R	R	U	R	U	U	U	U	U	N	N
D63 (27)	N	U	N	U	U	PI	U	U	N	N	N	N

D65 (26).....	U	U	PI	PI	U	U	R	R	U	U	R	R
D66 (27).....	U	PI	U	U	N	N	N	U	U	U	N	N
D67 (29).....	N	N	N	U	PI	PI	N	PI	N	U	N	PI
D68 (29).....	U	U	N	U	U	PI	N	PI	U	U	R	R
D74 (19).....	N	U	N	U	U	N	U	U	PI	U	N	N
D76 (39).....	PI	U	N	N	N	U	U	N	PI	U	N	N
D78 (35).....	U	U	U	U	PI	U	U	U	PI	U	N	N
D79 (34).....	U	U	PI	U	N	PI	N	U	PI	U	N	N
D81 (35).....	N	U	U	U	N	U	NT	PI	N	N	N	N
D89 (24).....	PI	U	N	N	U	N	U	U	PI	U	N	U
D90 (38).....	N	U	PI	U	N	U	U	PI	U	N	N	U
D91 (29).....	N	N	U	U	U	U	PI	U	U	N	N	N
D93 (48).....	U	U	U	U	U	N	U	N	PI	U	N	U
D94 (25).....	U	U	R	U	R	R	PI	U	U	U	R	R
D95 (38).....	U	U	U	R	R	R	R	R	U	U	N	U
D98 (34).....	U	R	R	U	N	U	N	PI	U	N	N	N
D99 (36).....	U	U	PI	N	PI	U	N	U	U	U	N	N
D101 (30).....	PI	U	N	U	N	U	U	N	U	U	N	N
D105 (33).....	N	U	U	U	U	N	PI	U	PI	U	N	U
D111 (38).....	U	N	PI	U	U	R	R	U	N	N	N	U
D112 (36).....	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	N	NT
D113 (40).....	U	NT	N	NT	U	NT	U	N	PI	U	N	NT
D115 (36).....	U	N	U	U	U	U	U	U	N	N	N	N
D121 (34).....	N	U	N	U	U	U	N	U	N	U	N	N
D123 (36).....	U	U	N	U	PI	N	U	U	N	R	R	R
D126 (38).....	N	PI	N	U	U	NT	U	U	N	N	N	N
D129 (29).....	U	U	U	U	U	PI	N	N	PI	U	N	U
D130 (37).....	U	U	N	U	U	NT	NT	PI	U	U	N	N
D131 (39).....	PI	U	U	N	R	NT	R	U	R	U	R	U
D132 (26).....	PI	U	R	N	U	U	NT	PI	PI	U	R	U
D133 (26).....	U	U	U	U	PI	U	PI	U	U	N	N	N
D134 (35).....	N	N	PI	U	PI	U	U	N	N	U	N	PI
D137 (28).....	U	U	PI	U	U	U	N	U	U	U	N	U
D139 (36).....	U	U	PI	U	U	U	U	PI	U	N	N	N
D140 (33).....	N	U	U	U	R	U	R	U	R	U	R	R
D141 (21).....	U	U	U	R	U	NT	R	U	R	U	N	U
D142 (29).....	N	NT	U	U	NT	NT	U	PI	U	NT	N	U
D144 (28).....	N	U	U	U	U	NT	N	U	U	U	N	N
D145 (28).....	U	U	R	U	U	NT	U	NT	PI	PI	R	R
D150 (31).....	PI	NT	NT	U	PI	NT	N	N	PI	N	N	U
D151 (38).....	N	NT	NT	N	PI	PI	U	U	PI	U	N	NT
D153 (25).....	PI	R	R	U	U	R	R	U	U	U	N	U
D159 (38).....	PI	N	N	U	N	U	U	U	U	U	N	U
D167 (24).....	N	NT	NT	U	U	U	U	NT	PI	U	N	U
D168 (30).....	U	U	PI	U	U	U	N	U	PI	U	N	U

[a] NT = not tested. All other abbreviations are as given in text.

Table 5

Maximum Likelihood Estimates of Both Recombination Fraction and Associated Lod Scores, for Each Pairwise Combination of Genetic Markers

	Normal Female		Maternal Trisomy 21	
Locus-1, Locus-2	Lod Score	Recombination Fraction	Lod Score	Recombination Fraction
D21S16/S13E, D21S110	10.184	.054	.788	.106
D21S16/S13E, D21S1	5.983	.229	2.040	.065
D21S16/S13E, SOD1	.285	.263	.104	.193
D21S16/S13E, D21S58	.071	.361	1.138	.083
D21S16/S13E, D21S17	.503	.288	.514	.123
D21S16/S13E, D21S55	.142	.351	.582	.160
D21S16/S13E, ETS2	.526	.361	.418	.166
D21S16/S13E, D21S15	.078	.401	.018	.298
D21S16/S13E, D21S19	.869	.210	.563	.151
D21S16/S13E, D21S112/S113/CD18	.000	.500	.967	.174
D21S16/S13E, COL6A1	. . .	. . .	.421	.216
D21S110, D21S1	5.774	.221	3.001	.000
D21S110, SOD1	.178	.402	.176	.000
D21S110, D21S58	.039	.262	.915	.093
D21S110, D21S17	.000	.500	.134	.202
D21S110, D21S55	.569	.160	.378	.183
D21S110, ETS2	.136	.437	.465	.000
D21S110, D21S15	.000	.500	.975	.000
D21S110, D21S19	.276	.271	.275	.192
D21S110, D21S112/S113/CD18	.000	.500	.672	.161
D21S110, COL6A1	.339	.380	.197	.227
D21S1, SOD1	4.219	.162	.272	.145
D21S1, D21S58	3.357	.160	1.860	.060
D21S1, D21S17	1.977	.292	.978	.130
D21S1, D21S55	3.136	.210	.415	.199
D21S1, ETS2	2.615	.287	.031	.304
D21S1, D21S15	.067	.452	.420	.133
D21S1, D21S19	.692	.331	.075	.277
D21S1, D21S112/S113/CD18	.628	.341	.578	.242
D21S1, COL6A1	.000	.500	.135	.285
SOD1, D21S58	1.768	.000	.263	.160
SOD1, D21S17	1.647	.107	.732	.000
SOD1, D21S55	.765	.252	.236	.175
SOD1, ETS2	.369	.271	.150	.203
SOD1, D21S15	1.672	.000	.007	.300
SOD1, D21S19	1.873	.080	.352	.000
SOD1, D21S112/S113/CD18	1.120	.140	. . .	. . .
SOD1, COL6A1	.010	.410	.483	.000
D21S58, D21S17	1.112	.060	1.754	.000
D21S58, D21S55	1.777	.090	2.741	.000
D21S58, ETS2	1.860	.150	.736	.000
D21S58, D21S15	1.771	.200	1.575	.000
D21S58, D21S19	.579	.242	.409	.143
D21S58, D21S112/S113/CD18	. . .	. . .	1.888	.116
D21S58, COL6A1	. . .	. . .	1.245	.121
D21S17, D21S55	1.558	.160	2.690	.000
D21S17, ETS2	3.981	.122	1.641	.000
D21S17, D21S15	4.231	.159	.210	.160
D21S17, D21S19	1.973	.070	. . .	. . .
D21S17, D21S112/S113/CD18	2.907	.200	. . .	. . .
D21S17, COL6A1	.297	.302	. . .	. . .

(*continued*)

Table 5 (continued)

Locus-1, Locus-2	Normal Female		Maternal Trisomy 21	
	Lod Score	Recombination Fraction	Lod Score	Recombination Fraction
D21S55, ETS2	8.336	.020	1.518	.070
D21S55, D21S15	2.090	.110	1.260	.081
D21S55, D21S19	2.089	.155	.100	.258
D21S55, D21S112/S113/CD18	. . .	. . .	.646	.223
D21S55, COL6A1	. . .	. . .	2.290	.081
ETS2, D21S15	6.845	.068	.843	.000
ETS2, D21S19	3.140	.130	.664	.104
ETS2, D21S112/S113/CD18	4.345	.170	1.124	.146
ETS2, COL6A1	1.815	.281	.783	.138
D21S15, D21S19	.970	.220	.494	.127
D21S15, D21S112/S113/CD18	5.755	.160	2.197	.090
D21S15, COL6A1	.000	.500	1.691	.072
D21S19, D21S112/S113/CD18	. . .	. . .	2.473	.049
D21S19, COL6A1	. . .	. . .	2.206	.055
D21S112/S113/CD18, COL6A1	4.254	.231	7.537	.038

tively large proportion (25 of 104) of our cases involved spontaneous or therapeutic abortions. If chromosome 21 is imprinted, it is at least formally possible that the parental origin of trisomy affects the likelihood that trisomy 21 conceptuses will survive to term. However, this is unlikely to explain our results, since the majority (79 of 104) of our cases involved live-born Down syndrome individuals and since, in this population as well, we observed a low (6.6%) level of paternal nondisjunction. In addition, it is difficult to understand how our live-born series would be any more biased toward maternal nondisjunction than were the earlier studied populations. Many of our live-born trisomic individuals were ascertained from "convenient" sources such as parent support groups; possibly this will artificially increase the proportion of maternal errors, but, as this ascertainment method was also commonly used in the cytogenetic studies, it is unlikely to explain the difference between the present and previous reports. Furthermore, the mean maternal ± SD age for our 79 live-born Down syndrome families was 30.2 ± 5.2 years, not significantly different from the value of 30.5 years reported by Juberg and Mowrey (1983) in their summary of 30 cytogenetic studies of trisomy 21 nondisjunction.

Second, the discrepancy may simply reflect the fact that we have studied a relatively small number of Down syndrome families with DNA markers and that, as more data accumulate, the proportion of paternally derived cases will increase. However, this explanation also seems unlikely. In the only other large, DNA-based study of trisomy 21 nondisjunction, Antonar-

Table 6

Maternal Age Distribution, by Status of Most Proximal Marker, in Trisomies of Maternal Origin

Most Proximal Marker	Total No. of Cases	No. in Maternal Age Group					Mean ± SD Maternal Age (years)
		<25 years	25–29 years	30–34 years	35–39 years	>39 years	
N: "MI"	35	4	8	10	12	1	32.0 ± 5.2
R: "MII"	11	2	4	4	1	0	28.3 ± 4.2

Table 7

Number of Detectable Crossovers, by Status of Most Proximal Marker, in Trisomies of Maternal Origin

Most Proximal Marker	Total No. of Cases	No. of Crossovers			Mean ± SD No. of Crossovers
		0	1	2	
N: "MI" ...	36	26	8	2	.33 ± .59
R: "MII" ...	11	2	7	2	1.00 ± .63

akis et al. (1991) observed a paternal nondisjunction rate of 4.7% (nine of 192 cases). Combining their results with those of the present study gives a paternal error rate of 5.1%, a value highly significantly reduced from expected values of 20% ($\chi_1^2 = 40.4$, $P < .001$) or even 10% ($\chi_1^2 = 7.7$, $P < .01$).

Third, it may be that the discrepancy between the cytogenetic and molecular studies is real and that it is attributable to imprecision in the cytogenetic technique. There are several reasons for making this suggestion. The cytogenetic technique is subjective and involves discrimination of variants which may be similar in size or staining intensity; the approach is limited to examination of variation in the short arm or pericentromeric region of chromosome 21 and, unlike DNA analyses, cannot be verified with other markers; and, finally, the error rate found in the present study supports our contention, since two of the 25 cytogenetic determinations of parental origin were apparently misclassified. Subsequent reexamination of these two cases revealed only minor differences between the paternal and maternal chromosome 21 heteromorphisms, results compatible with misclassifications of parental origin. We suggest that similar overinterpretations are not uncommon in other cytogenetic studies of chromosome 21 nondisjunction and that these have artificially increased the apparent rate of paternal nondisjunction associated with trisomy 21.

Thus, we conclude that paternal nondisjunction accounts for approximately 5%–6% of trisomy 21 cases and that previous cytogenetic studies have overestimated the contribution of paternal errors. If this interpretation is correct, it casts doubt on several hypotheses which are based on the cytogenetic data. For example, in the cytogenetic studies there was little evidence for differences in the maternal ages in trisomies of maternal or paternal origin, leading to the idea that the maternal age effect in trisomy derives from a decreased likelihood of abortion of trisomic conceptuses in older women, i.e., the so called "relaxed selection" hypothesis (Aymé and Lippman-Hand 1982). This idea now must be reexamined using data from DNA markers. Similarly, cytogenetic studies reporting an increased incidence of either specific environmental exposures (e.g., maternal irradiation [Aymé et al. 1986]) or predisposing genetic factors (e.g., double NORs [Jackson-Cook et al. 1985] and thyroid antibodies [Flannery and Jackson-Cook 1986]) in the parent of origin also must be reconsidered.

Our interpretation also casts doubt on the utility of chromosome heteromorphism analysis. However, we think it premature to dispense with the technique and suggest that it still can play a useful role in many situations. For example, in analyses involving the entire genome, in which it is possible to compare results at different polymorphic regions, the cytogenetic approach should be quite reliable. This will include (1) analyses of the parental origin and/or stage of origin of hydatidiform moles, triploids, tetraploids, and ovarian teratomas and (2) determinations of the proportion of host and donor cells after bone marrow transplants. In addition, for chromosomes such as chromosome 21, where highly polymorphic centromeric DNA markers are not yet available, judicious use of chromosome heteromorphisms will still be helpful. However, our experience suggests that it may be necessary to use multiple banding techniques (e.g., NOR staining, Q-banding, fluorescent in situ hybrid-

Table 8

Maternal Age Distribution, by Number of Detectable Crossovers in Cases of Presumptive Maternal Meiosis I Origin

No. of Crossovers	Total No. of Cases	No. in Maternal Age Group					Mean ± SD Maternal Age (years)
		<25 years	25–29 years	30–34 years	35–39 years	>39 years	
0	25	4	7	5	8	1	31.4 ± 5.8
1	8	0	1	5	2	0	32.5 ± 2.7
2	2	0	0	0	2	0	37.0 ± 1.4

ization with probes to repetitive sequences [e.g., see Waye and Willard 1990]) and that it is essential to conservatively score only those chromosomes having extreme variants.

Recombination and Nondisjunction

In our studies of recombination in five paternally derived trisomies, we observed crossing-over in three cases, and, in the other two, paternal heterozygosity was reduced to homozygosity at all informative loci. Thus, failure to pair and/or recombine at meiosis I cannot be the mechanism responsible for any of the five trisomies of paternal origin.

For the 77 maternally derived trisomies, we detected recombination in 25 cases, and in four other cases all loci were reduced to homozygosity. In the remaining 48 cases, heterozygosity was maintained at all loci, implying an association between reduced recombination and maternal meiosis I nondisjunction. This was confirmed by the centromere mapping studies, which demonstrated a threefold reduction in map length by comparison with the conventional chromosome 21 map.

Thus, our results are consistent with the suggestion of Warren et al. (1987) that failure to recombine is important in the genesis of trisomy 21. However, it is not yet possible to estimate the magnitude of this effect, for several reasons. First, the majority of cases were not informative for all loci along 21q, and therefore not all recombinants will be detected. In fact, for several cases only one or two loci were informative. Furthermore, we cannot formally exclude the possibility that, in our cases, recombination occurred distal or proximal to the set of markers we studied. Second, without a reliable centromeric polymorphism, it is not possible to determine which trisomies derive from meiosis I errors and which derive from meiosis II errors; presumably only the former are associated with aberrant recombination. We attempted to score trisomies as being of meiosis I or II origin on the basis of information on nonreduction/reduction at proximal 21q markers. We identified a significant decrease of detectable crossovers for the meiosis I group, suggesting that we were able, with some accuracy, to discriminate between the two meiotic stages. However, some of these cases may have been misclassified because of crossing-over between the centromere and the proximal markers. Recent studies of CEPH families have suggested that chromosome 21 alphoid sequences are approximately 6 cM from D21S13E (Jabs et al. 1991), and, while our studies would indicate that this region is shorter in trisomy-generating meioses than in normal meioses, a small proportion of our meiotic assignments may be in error.

Because of these uncertainties, it is unlikely that all 48 of the maternally derived trisomies with no detectable exchanges were due to recombination failure. Nevertheless, it now seems reasonable to conclude that a significant proportion of maternal meiosis I nondisjunction of chromosome 21 is associated with failure to recombine. If so, this could be due to failure of synapsis, to abnormalities in synapsis which prevent recombination, or to abnormalities in recombination after normal synapsis. Our assay cannot distinguish between these possibilities, and there is little evidence bearing on this question. However, in a recent electron-microscopic analysis of pairing in oocytes from chromosomally normal female fetuses, Speed (1989) observed synaptic errors in nearly half (46%) of 1,200 oocytes. As synaptic errors are much less frequent in the human male (Speed 1989), it may be that the human female is particularly susceptible to errors at this stage of meiosis. If true, this suggests that failure to recombine is simply a by-product of an earlier occurring abnormal process.

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