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Human Molecular Genetics, 1998, Vol. 7, No. 8 **1221–1227**

Elucidating the mechanisms of paternal non-disjunction of chromosome 21 in humans

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Received February 10, 1998; Revised and Accepted April 29, 1998

Paternal non-disjunction of chromosome 21 accounts for 5–10% of Down syndrome cases, therefore, relative to the maternally derived cases, little is known about paternally derived trisomy 21. We present the first analysis of recombination and non-disjunction for a large paternally derived population of free trisomy 21 conceptuses ($n = 67$). Unlike maternal cases where the ratio of meiosis I (MI) to meiosis II (MII) errors is 3:1, a near 1:1 ratio exists among paternal cases, with a slight excess of MII errors. We found no paternal age effect for the overall population nor when classifying cases according to stage of non-disjunction error. Among 22 MI cases, only five had an observable recombinant event. This differs significantly from the 11 expected events ($P < 0.02$, Fisher's exact), suggesting reduced recombination along the non-disjoined chromosomes 21 involved in paternal MI non-disjunction. No difference in recombination was detected among 27 paternal MII cases as compared with controls. However, cases exhibited a slight increase in the frequency of proximal and medial exchange when compared with controls (0.37 versus 0.28, respectively). Lastly, this study confirmed previous reports of excess male probands among paternally derived trisomy 21 cases. However, we report evidence suggesting an MII stage-specific sex ratio disturbance where 2.5 male probands were found for each female proband. Classification of MII cases based on the position of the exchange event suggested that the proband sex ratio disturbance was restricted to non-telomeric exchange cases. Based on these findings, we propose new models to explain the association between paternally derived trisomy 21 and excessive male probands.

INTRODUCTION

Trisomy 21 accounts for >95% of Down syndrome (DS) (1). Most trisomy 21 cases are the result of a maternal meiotic non-disjunction event and, thus, studies of the cause of non-disjunction have focused primarily on the maternal cases. Paternal non-disjunction of chromosome 21 accounts for 5–10% of all trisomy 21 (2,3). Therefore, relatively little is known about the cause of non-disjunction in the paternally derived cases.

Molecular studies determining the parent and stage of origin of trisomic conceptuses indicate remarkable variation among chromosomes in the frequency of paternally derived trisomies. For example, paternally derived errors account for 100% of 47,XYYs and ~40–50% of 47,XXYs and trisomy 2 conceptuses (4,5). However, they account for only a small proportion (5–15%) of 47,XXX and most autosomal trisomies, and rarely have been identified among trisomy 16 fetuses, as reviewed by Hassold (5). This variation in the occurrence of paternally derived trisomies may be the result of chromosome-specific variation in mechanisms of paternal meiotic non-disjunction. Two alternate explanations have been proposed (5). First, it may be that the proportion of paternal non-disjunction among different chromosomes is in fact similar but, depending on the frequency of maternal non-disjunction, the perceived paternal contribution will vary. Second, genomic imprinting may influence the likelihood of survival of paternal or maternal trisomic conceptuses for specific chromosomes.

Only one previous study combining cytogenetic and molecular tools to examine paternal non-disjunction of chromosome 21 has been reported (6). This study found twice as many meiosis II (MII) cases as meiosis I (MI), which contrasted with the maternal cases where an MI case is nearly three times as likely as an MII. Additionally, the study reported an increase in paternal age which was restricted to MI non-disjunction. Finally, they confirmed the

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observation of an altered sex ratio favoring male probands in DS, particularly among the paternally derived trisomy 21 cases (7–10). The reason for this excess of male probands is unknown. However, a recent study of disomy 21 sperm showed an excess of Y-bearing sperm, suggesting that the effect may be particularly due to the mechanism of paternal non-disjunction (11). Clearly, some mechanism exists which skews both the male gametic sex ratio among disomy 21 sperm as well as the sex ratio observed among trisomy 21 live births. Because the altered sex ratio is seen only with free trisomy 21 cases and not with translocations (12), models explaining the phenomenon often include the non-disjoined chromosomes 21 segregating with the Y chromosome.

Recent studies of aneuploid conceptuses indicate an association between aneuploidy and altered recombination patterns among both paternal and maternal errors. For example, paternally derived cases of 47,XXY show an association between reduced recombination and errors at MI, with an overall reduction in genetic map length from 52 cM for the normal map to only 13 cM for the trisomic map (4). Maternal errors involving both the sex chromosomes and the autosomes (i.e. chromosomes 15, 16, 18 and 21) also demonstrate reduction in the genetic map lengths built for the trisomic populations as compared with normals (13). Reduced recombination has been shown to be due to both a complete lack of recombination in a proportion of cases as well as to a reduced number of exchanges. For example, it is estimated that nearly 70% of all paternal 47,XXY cases result from a meiosis where the XY bivalent fails to pair and/or recombine (4). Similarly, ~40% of maternal MI trisomy 21 cases are inferred to be achiasmate (14). These findings are in sharp contrast to the normal situation, where an obligatory crossover(s) is observed and is involved in proper disjoining of a bivalent (13).

Alteration in the placement of the recombination event in both maternal MI and MII errors of chromosome 21 has also been observed (15). Among normal female meiotic events, recombination is relatively uniform along the length of the chromosome. In contrast, among maternal MI errors, the recombinant event is usually telomeric, whereas among maternal MII errors, the event is pericentromeric. The unexpected finding of an association between MII errors and altered recombination suggested that, at least in females, essentially all meiotic errors are initiated during MI (see Discussion). However, the possibility of a true MII non-disjunction has not been ruled out, and such an error cannot be distinguished from an MI-derived MII case (MI/MII).

The purpose of the present study was to analyze a large population of paternally derived trisomy 21 cases to further examine factors associated with non-disjunction. This study updates the analysis of Petersen *et al*. (6) and presents the first analysis of the recombination profile for such cases. Given that altered recombination has been found in both paternally derived 47,XXY cases and maternally derived trisomy 21 cases, it is of interest to determine if paternally derived cases of trisomy 21 also show an association with altered recombination. Extending molecular studies beyond origin and stage assignments to include such an analysis of recombination events provides additional information to help elucidate mechanisms of paternal non-disjunction of chromosome 21.

RESULTS

Stage of origin and paternal age

Of the 67 cases, 22 were consistent with MI non-disjunction and 39 with MII (Table 1). Stage of origin could not be determined for the remaining six cases as they were uninformative at the pericentromeric markers. However, crossover(s) were identified in four of these six cases, which indicated meiotic origin. Further examination of the 39 cases consistent with MII non-disjunction indicated that 28 were truly meiotic in origin as a recombinant event was observed along 21q. Of the remaining 11 cases, 10 were considered mitotic in origin as they were reduced to homozygosity at all informative loci. The remaining case was reduced at all informative loci but had no informative loci in the telomeric interval; therefore, it was not assigned a stage of error (MII versus mitotic). The ratio of MI to MII errors among these paternal cases was 0.79. We found no significant difference between the mean paternal age of the 57 paternally derived meiotic cases (29.5 ± 6.7 years) and the mean paternal age of controls (30.3 ± 6.6 years). Furthermore, when subdividing the overall population of cases according the stage of error, no significant differences were observed among any of the groups: for MI, MII and mitotic cases, the mean paternal ages were 29.2 ± 7.8, 28.8 ± 5.7 and 31.8 ± 5.7 years, respectively.

Table 1. Comparison of stage of non-disjunction error among paternal and maternal cases

Stage of error	Parent of origin	
	Paternal	Maternal
MI	22	398
MII	28	137
Mitotic	10	11
MI:MII	0.79[a]	2.90

[a]Significantly different from maternal population, $P < 0.001$ (Fisher's exact).
Note: includes only cases with known origin of non-disjunction error.

Altered recombination among paternal trisomy 21 cases

We examined the recombination patterns among the 22 MI and 27 MII paternal cases (one MII case was excluded as it was not informative for recombination status in at least four of the five intervals outlined in the inclusion criteria under Materials and Methods). Based on the genetic length of the normal male chromosome 21 map [48 cM (16)], one exchange is assumed for each tetrad. Thus, 11 crossovers were expected to be observed among the 22 MI cases, as an exchange product is detected only half the time per tetrad. We found only five crossovers among the 22 MI cases, ($P < 0.02$, Fisher's exact). This observation mirrors the reduction in recombination observed among the maternal MI cases of trisomy 21 (15).

Next we examined the amount and placement of recombination among the MII cases. By definition, each of the 28 MII cases had at least one detectable recombinant event, as those with no

observable crossovers were excluded as mitotic errors (see Materials and Methods). No cases had more than one recombinant event. As one exchange is expected along chromosome 21 in normal male meiosis, our MII population did not differ from normals in the amount of recombination. The placement of exchange, another possible risk factor, was examined by counting the observed recombinants and then inferring the underlying exchanges for the 27 cases meeting the inclusion criteria (see Materials and Methods). Cases and controls showed no differences in the frequency of exchange occurring on an interval-by-interval basis nor in the overall distribution of exchanges (Table 2). However, cases exhibited a slight increase in the amount of exchange occurring in the three most proximal intervals when compared with controls (0.37 versus 0.28, respectively).

Table 2. Exchange distribution among paternal MII cases

Interval location[a]	Paternal MII	Controls[b]
1 [*D21S369–D21S1*]	0.13	0.08
2 [*D21S214–D21S210*]	0.11	0.10
3 [*D21S82–D21S213*]	0.13	0.10
4 [*D21S224–IFNAR*]	0.07	0.15
5 [*D21S17–D21S1575*]	0.56	0.55

[a]Beginning with most centromeric interval as described under Materials and Methods.
[b]Exchange frequency estimated from observed recombinants on chromosome 21 from normal males and does not include the infrequent double exchanges (27).

Table 3. Sex ratios among paternal cases

Stage	Male probands	Female probands	Sex ratio
MI	10	12	0.83
MII	20	8	2.50[a]
MI or MII	4	0	–
Mitotic	5	5	1.00
Overall meiotic	34	20	1.70[a]

[a]Significantly different from normal sex ratio (1.06, male:female), $P < 0.05$, Fisher's exact.

Meiosis II-specific altered sex ratio

The sex ratio (male:female) for the meiotic cases of our population was 1.70, which is significantly different from the normal sex ratio of 1.06 among Caucasian live births (17) ($P < 0.04$, Fisher's exact) confirming previous reports (6–10). Surprisingly, our data indicated that the association was specific to cases consistent with MII errors. The sex ratio among MI cases was 0.83, while the MII sex ratio was 2.50 (Table 3).

Exchange placement and the sex ratio

There was no significant difference between the exchange distributions of the MII cases and normals, but there was a slight increase in the amount of proximal–medial exchange among the MII cases. Because the majority of exchanges occur telomerically in normal male meiotic events, we further classified the MII population as cases with either a proximal–medial exchange (intervals 1–3) or a distal–telomeric exchange (intervals 4 and 5) and re-examined the sex ratio disturbance. For the 17 cases with a telomeric exchange, there were 10 male and seven female probands, nearly a 1:1 ratio. Surprisingly, among the 10 cases with a proximal–medial exchange, only one was a female (Table 4).

Table 4. Association between exchange placement and sex ratio[a]

Interval of exchange location	Probands Male	Probands Female	Sex ratio
Proximal–medial (intervals 1–4)	9	1	9.0[b]
Telomeric (interval 5)	10	7	1.43

[a]One case is excluded based on the inclusion criteria outlined in Materials and Methods.
[b]Significantly different from normal sex ratio (1.06, male:female), $P < 0.001$, Fisher's exact.

DISCUSSION

By combining 46 'new' cases with the 21 'shared' cases from the study of Petersen *et al.* (6), tripling the number of MI cases and doubling the MII cases available for study, we further examined factors associated with paternal non-disjunction of chromosome 21. This larger data set allowed us to re-examine the findings of Petersen *et al.* and more confidently interpret results. Second, we investigated the association between altered recombination along chromosome 21 and paternal non-disjunction, a phenomenon observed for many other trisomic conditions.

Stage of origin and paternal age

The present study confirms the report of Petersen *et al.* (6) of an excess of MII errors among the paternally derived trisomy 21 cases, although the difference is not as large as the earlier study suggested [MII:MI = 2.14, Petersen *et al.* (6) and 1.27, present report]. These findings are in contrast with the maternal population, where an MI error is nearly three times as likely as an MII error. We assume that this reflects differences between the meiotic process in males and females (e.g. the timing of MI and MII in spermatogenesis versus oogenesis, differences in recombination profiles among males and females) and/or different mechanisms of surveillance and segregation of susceptible tetrads.

Petersen *et al.* (6) found a paternal age effect limited to the seven MI cases and noted in their report a need for more cases to confirm this finding. With 22 MI cases, an age effect is no longer observed. However, these numbers are still limited in their power to detect a paternal age effect. Because of the difficulty of accruing large numbers of paternal trisomic cases, an alternative approach of sperm studies may prove to resolve this question more accurately.

Recombination studies

This study is the first to report an association between altered recombination along chromosome 21 and paternal non-disjunction. The data from the MI cases suggest an association with a reduction in recombination due to a complete lack of

recombination similar to the paternal XXY cases (4). The reduction in recombination associated with the XY bivalent may be attributable to the MI pairing configuration where only a single chiasma is usually formed, with the loss of this obligatory chiasma resulting in non-disjunction at anaphase I (4). Our finding of reduced recombination among the MI paternal cases of trisomy 21 suggests that the chromosome 21 bivalent in males may non-disjoin at anaphase I for the same reason as the XY bivalent; i.e. the sensitivity to reduced recombination results from the susceptibility created by normally having only one chiasma.

When examining the MII cases, no significant difference either in the amount or placement of exchange was observed compared with controls. A slight increase in the amount of proximal–medial exchange was observed compared with telomeric exchange. This observation may be important when considering that in normal male meiosis most exchange occurs in distal 21q (Table 2). More cases are required to confirm this finding.

Sex ratio among paternal trisomy 21 cases

Finally, this larger study population confirms earlier reports (6–10) of the altered sex ratio favoring male conceptuses (1.70, male:female). Maternal cases also have a slight excess of male probands, with a sex ratio equal to 1.22. Griffin *et al*. reported that the gametic sex ratio was disturbed with a ratio of 1.57 Y-bearing to X-bearing disomy 21 sperm (11). Given that paternal cases account for <10% of trisomy 21 cases, it seems that both alterations in the primary sex ratio of male gametes and selection against trisomy 21 female conceptuses must be operating to result in the sex ratio disturbance seen among the DS live born population. Huether *et al*. (18) found no evidence of a selection against trisomy 21 female conceptuses when comparing sex ratios at gestational ages <16 weeks, >16 weeks and among live-borns. Therefore, the putative selection against female trisomy 21 conceptuses must be occurring very early (i.e. before 9 weeks gestational age).

Perhaps the most unexpected result was the evidence of an MII-specific sex ratio disturbance. Given that exchange typically occurs in distal 21q in normal male meiosis and that placement of exchange has been shown to be a risk factor for non-disjunction, we speculated that the sex ratio disturbance might be related to proximal–medial exchange in our MII cases. Therefore, we further examined the MII population according to distal–telomeric or proximal–medial exchange and found an even stronger sex ratio disturbance. The 17 cases with a distal–telomeric exchange had nearly a 1:1 sex ratio while the 10 cases with a proximal–medial exchange had a 9:1 sex ratio. These findings suggest that the sex ratio disturbance is not only specific to MII cases, but to meiotic events involving a proximal–medial exchange on chromosome 21.

Two models explaining the sex ratio disturbance were outlined in Petersen *et al*. (6). The first model proposes the idea of an achiasmate pairing system, similar to that in *Drosophila* females (19). This model hypothesizes an abnormal MI where both the sex chromosomes and chromosomes 21 fail to pair. The absence of chiasma would allow the large X chromosome to segregate from the non-disjoined chromosomes 21 in an effort to balance the genetic material of the cell, resulting in a disomy 21 Y-bearing sperm. The second explanation involves an aberrant exchange event between the Y chromosome and a chromosome 21 leading to a physical association of the two non-homologous chromosomes. Failure to resolve the exchange might result in migration to the same pole. Assuming random segregation of the X and the other unpaired chromosome 21, one result would be a Y-bearing sperm disomic for chromosome 21. Both models predict no homologous recombination between the chromosomes 21; however, we found that >50% of the paternal cases had one observed recombinant. Thus, new models that consider recombination between the homologs as a potential player in the sex ratio disturbance must be proposed.

Considering these findings in light of recent work on maternal MII cases where pericentromeric exchange has been shown to be a susceptibility factor for non-disjunction to occur (15), we postulate that perhaps a proximal–medial exchange creates the same susceptibility in male meiosis. Attempts to resolve such chiasma may result in two possible non-disjunction products as outlined in Lamb *et al*. (15). The non-disjunction product after MI may be either an entangled tetrad or an unbalanced product of a whole chromosome plus a half chromosome resulting from precocious separation of the sister chromatids (PSSC). Recent studies of oocytes rejected from *in vitro* fertilization attempts would suggest that the latter may be the more likely outcome (20). Under either scenario, if PSSC or entanglement at MI is followed by an unscheduled reductional division at MII, one end result would be a disomic gamete with both chromatid contributions from the same homolog (Fig. 1). This product would be classified as an MII error like the MI/MII class among maternal cases. One important prediction of either model is an imbalance of genetic mass at the MI metaphase. It is an intuitively attractive idea to imagine the cell, when faced with an imbalance of chromosome material during male meiosis, segregating the non-disjunction product to the less crowded pole harboring the Y chromosome. The premise for this idea is derived from the 'spindle pole crowding model' implicated in a role for the segregation of heterologous achiasmate chromosomes as described by Hawley and Therkauf (19). Rather than serving as a distributive system for achiasmate chromosomes, it serves as a surveillance system for balancing chromosome mass between the division products involving non-disjunction of susceptible tetrads.

This model requires one to reconcile the lack of a sex ratio disturbance among the MI cases, where under the classic model of MI non-disjunction, both homologs migrate to the same pole. If such a system existed where unbalanced genetic mass was shuttled to the less crowded pole, the MI cases should also exhibit a disturbance in the sex ratio. We suggest two possible explanations. First, perhaps the surveillance system is only alerted by the tension resulting when a chiasmate bivalent is on the spindle. About 50% of the paternal MI cases are inferred to be achiasmate and would not be involved in this surveillance system. Alternatively, as suggested by Angell (20), the majority of MI non-disjunction may occur as the result of PSSC. In the case of achiasmate homologs, both homologs may line up individually on the MI plate as they are not tethered by a chiasma. Under this scenario, they simultaneously would undergo PSSC, resulting in the more common balanced non-disjunction product of 22 whole chromosomes plus one chromatid from each chromosome 21. The cell would not be presented with imbalanced genetic mass and a 1:1 sex ratio would be expected (Fig. 2).

Regardless of the detailed mechanism of non-disjunction and its association with sex ratio disturbances, our data suggest that exchange patterns play a role in paternal non-disjunction of chromosome 21 in a similar manner to that seen among the

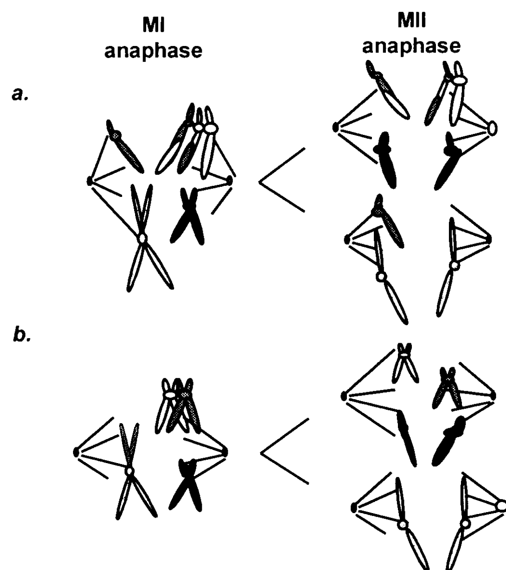

Figure 1. Models illustrating the association between the Y chromosome and non-disjoined recombinant chromosomes 21 (black, Y chromosome; small white and gray, chromosome 21 homologs; large white, X chromosome). (**a**) PSSC of recombinant chromosomes 21 resulting in imbalanced non-disjunction products where the larger of the non-disjunction products segregates with the Y chromosome against the larger X chromosome in an effort to balance the chromosome mass in the dividing gamete. Following a reductional division of the imbalanced non-disjunction product at MII, end products of meiosis include: a disomy 21, Y-bearing sperm with both chromosomes from same homolog; a normal X- and Y-bearing sperm; and one X-bearing sperm nullisomic for chromsome 21. (**b**) Imbalance of chromosome mass resulting from entanglement of recombinant chromosomes 21. The entangled tetrad migrates to the less crowded pole harboring the Y chromosome at MI anaphase. A reductional division at MII results in two disomy 21, Y-bearing sperm and two X-bearing sperm nullisomic for chromosome 21.

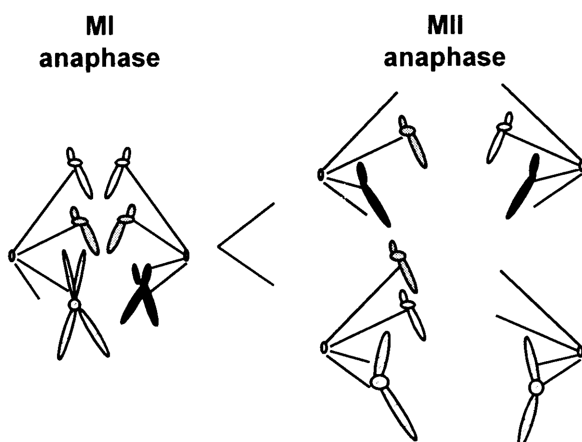

Figure 2. The absence of a chiasma may allow PSSC of both chromosome 21 homologs at MI anaphase (black, Y chromosome; small white and gray, chromosome 21 homologs; large white, X chromosome); random segregation of the chromosomes 21 could result in either normal X- and Y-bearing sperm (top) or X- and Y-bearing sperm disomic for chromosome 21 with a chromosome from each homolog (bottom). [Adapted from Angell (19).]

maternal cases. A 'two hit' model has been proposed to explain the association between altered recombination and the maternal cases (15). The first hit is the creation of a tetrad susceptible to non-disjunction as a result of its exchange configuration. As the exchange patterns are not associated with maternal age, the second hit is thought to be attributed to age-related factors, although no specific factors have been clearly implicated. The meiotic arrest occurring in MI during oogenesis creates a target of between 10 and 40 years for the second hit to 'strike'. There is no such age-related arrest during spermatogenesis. This difference may be reflected in the different stage distributions of MI and MII errors among the maternal and paternal populations as well as in the increased frequency of maternal non-disjunction in general. The second hit among paternal cases is presently unclear, but may be similar to that occurring in younger women who have a DS conceptus (e.g. an environmental exposure).

Finally, it is interesting to note that the alterations in recombination observed among this paternally derived population of trisomy 21 cases are similar to the phenotype associated with *tam1* mutants in yeast (21). The Tam1 protein is meiosis-specific and localizes to the ends of meiotic chromosomes. *tam1* strains exhibit both homolog non-disjunction and PSSC, and have an increase in homologs which fail to receive a crossover. Possibly a human homolog of Tam1 might be defective in some of these paternal cases and, therefore, creates the observed susceptible tetrad configurations.

MATERIALS AND METHODS

Study population

Blood or tissue samples were collected from DS conceptuses and their parents during the past 8 years as part of an ongoing collaborative study of the etiology of trisomy 21 (2,22,23) For the present study, analyses were restricted to include only non-mosaic, paternally derived cases of free trisomy 21 as determined by cytogenetic analysis. Total sample size equaled 67 (out of 601 total free trisomy 21 cases). Of these 67 paternal cases, 21 were included in the sample of 36 paternally derived cases of trisomy 21 reported by Petersen *et al.* (6). Of the 21 'shared' cases, three were MI cases, 12 were MII cases, three were considered mitotic, and the remaining three had an unknown origin. Controls for determining paternal age were the fathers of all infants born in the Atlanta metropolitan area in 1996 (24).

DNA studies

DNA was extracted from blood or tissue samples as described (25), and chromosome 21 polymorphisms were detected using Southern blotting techniques or the polymerase chain reaction (PCR). The set of ordered markers from the centromere to the telomere of the long arm included: [*D21S369*, *D21S215*], [*D21S258*, *D21S120*], [*D21S13*, *D21S16*, *D21S192*], [*D21S11*, *D21S1*], [*D21S214*, *D21S232*], *D21S210*, *D21S213*, [*D21S223*, *D21S224*, *IFNAR*], [*D21S17*, *D21S167*], [*ETS2*, *D21S156*], *HMG14*, [*D21S212*, *D21S113*], [*D21S171*, *D21S1261*, *D21S19*, *COL6A1*], [*D21S1446*, *D21S1575*]. The 29 markers were reduced to 14 regions (as indicated by square brackets), with each region defined as a group of markers known to be tightly linked in controls and among which no recombination was observed in our trisomic set. In our analyses, we first determined the parent of origin of trisomy, with each conclusion based on results from at least two markers. Subsequently, we evaluated the stage of origin of trisomy by comparing chromosome 21 pericentromeric

markers of the parent who contributed the extra chromosome with those of the trisomic offspring. If parental heterozygosity was retained in the trisomic offspring ('non-reduction'), we concluded that the error occurred during MI, and if parental heterozygosity was reduced to homozygosity ('reduction'), we concluded that the error occurred during MII or was a mitotic error. As highly polymorphic chromosome 21 centromeric markers have not been identified so far, these determinations were based on the most proximal informative marker of the following regions: *D21S369–D21S192* (see order above). This means that some proportion of assignments will be in error, although physical mapping studies of proximal 21q (26) and linkage studies of controls (27) suggest that the error rate may not exceed 5–10%.

We distinguished MII from mitotic errors by evaluating other, non-pericentromeric loci. If a trisomic individual was reduced at all informative loci, including at least one each in a proximal region (*D21S369–D21S1*), medial region (*D21S214–D21S167*), distal region (*ETS2–D21S213*) and a telomeric region (*D21S171–D21S1575*), we inferred a post-zygotic, mitotic origin of trisomy. If the trisomic individual was non-reduced at one or more loci, we concluded that the error had an MII origin. Distinguishing mitotic cases from truly meiotic cases is crucial when asking questions pertaining to recombination. If a meiotic error is classified erroneously as mitotic and excluded from subsequent analyses, the amount of recombination is inflated. Conversely, if these cases are truly mitotic in origin and they are included in the analyses, recombination will be decreased erroneously. Although we cannot rule out the possibility that a proportion of the putative mitotic cases are truly meiotic errors of achiasmate chromosomes 21, two observations suggest that these are more likely to be mitotic. First, when examining our entire study population including maternal and paternal errors, we observe equal numbers of putative mitotics involving the maternal chromosome ($n = 10$) and the paternal chromosome ($n = 10$). Second, there is a 1:1 sex ratio among these putative mitotics. As a mitotic error is equally likely to involve either the maternal or paternal chromosome 21, as well as a male or female conceptus, these observations support the judgment to remove these cases from the meiotic analyses.

Inferring placement of exchange

The exchange distribution (i.e. events occurring at the four strand stage) for the MII subpopulation was estimated based on observed recombinant events recovered from chromatids. A detailed description of the method and CEPH genotype data analysis for the normal male map can be found in Lamb *et al*. (28). Briefly, this approach is a modification of Weinstein's method for studying tetrad exchange patterns in *Drosophila* (29,30). It involved dividing the chromosome into five roughly equivalent physical intervals, small enough to justify the assumption that no more than one crossover would be observed in any interval. Interval delineations, proximal to distal, were defined as follows: [*D21S369–D21S1*], [*D21S214–D21S210*], [*D21S82–D21S213*], [*D21S224–IFNAR*] and [*D21S17–D21S1575*]. Each interval was examined for the presence of a recombination event. If such an event occurred at the junction of two intervals, the recombination was recorded in both intervals with a probability of occurrence of 1/2. This allowed determination of the overall recombination pattern for each chromosome, which was then converted into frequencies for each class of recombination. Once these frequencies were known, the exchange distributions were estimated. For inclusion in the analysis, a case had to be informative in at least four of the five intervals including the telomeric interval. To test the hypothesis that the distributions are different, the obvious analysis would be a χ^2 test. However, use of that test would be incorrect because the proportions shown are estimated, not observed directly. Such a test would be strongly biased toward showing a difference. More sophisticated bootstrap or permutation methods are possible, but there are some unresolved technical issues in the correct application of them to these data, as discussed in Lamb *et al*. (28). Therefore, no statistical analyses on these particular data were performed.

ACKNOWLEDGEMENTS

S.L.S. wishes to thank Lori Griffin for the establishment of lymphoblastoid cell lines. The work of S.L.S. was supported by NIH PO1 HD 3322111 and US PHS-NIH-MO-1-RR00039 to the General Clinical Research Center. M.B.P.'s work was supported by E.C. BIOMED grants BENE-CT-0015 and BMH14-CT96–0544 to the European Chromosome 21 Consortium, Else og Mogens Wedell-Wedellsborgs Fond, Smedemester Niels Hansen og hustru Johanne f. Frederiksen's Legat, Brodene Hartmanns Fond, Fru C. Hermansens Mindelegat, Direktor Jacob Madsens & Hustra Olga Madsens Fond, Kirstine Fonden, Kong Christian den Tiendes Fond and Lily Benthine Lunds Fond.

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