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Genome-wide variation in recombination in female meiosis: a risk factor for non-disjunction of chromosome 21

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Altered recombination patterns along non-disjoined chromosomes is the first molecular correlate identified for non-disjunction in humans. To understand better the factors related to this correlate, we have asked to what extent is recombination altered in an egg with a disomic chromosome: are patterns limited to the non-disjoined chromosome or do they extend to the entire cell? More specifically, we asked whether there is reduced recombination in the total genome of an egg with a non-disjoined chromosome 21 and no detectable recombination. We chose this subclass of non-disjoined chromosomes to enrich potentially for extremes in recombination. We found a statistically significant cell-wide reduction in the mean recombination rate in these eggs with non-disjoined chromosomes 21; no specific chromosomes were driving this effect. Most importantly, we found that this reduction was consistent with normal variation in recombination observed among eggs. Thus, given that recombination is a multifactorial trait, these data suggest that when the number of genome-wide recombination events is less than some threshold, specific chromosomes may be at an increased risk for non-disjunction. Further studies are required to confirm these results, to determine the importance of genetic and environmental factors that regulate recombination and to determine their impact on non-disjunction.

INTRODUCTION

Non-disjunction occurs when chromosomes fail to segregate properly during meiosis. A non-disjunction error may result in aneuploid gametes that are disomic or nullosomic for the non-disjoined chromosome. Subsequent fertilization of these types of gamete results in trisomy or monosomy for the non-disjoined chromosome, and, most often, the aneuploid fetus is aborted spontaneously. Of the autosomal trisomies that do survive to term, most are mentally impaired. Thus, non-disjunction is the leading cause of pregnancy wastage and mental retardation in humans. In spite of this marked impact on public health, the actual cause of non-disjunction has not been determined. For many years, the only risk factor for non-disjunction was advanced maternal age. Although several models have been proposed to explain this age effect (1), it remains enigmatic. Recently, however, many studies using chromosome-specific molecular markers to examine the patterns of recombination along non-disjoined chromosomes have identified the first molecular correlate of non-disjunction in humans: altered recombination. Many studies have focused on this association and its possible implications for understanding the etiology of non-disjunction involving human chromosomes (2–10). The association between altered recombination and the non-disjunction of chromosome 21 is outlined below as it provides the basis for the present study.

Trisomy 21 is the most common trisomy among liveborns and is the chromosome abnormality responsible for >95% of individuals with Down syndrome (DS). Thus, it is one of the most extensively studied human non-disjunction events. In ~90% of trisomy 21 individuals, the additional chromosome is maternal in origin (4,11–13). Using pericentromeric markers to infer the meiotic stage of origin of this error, ~70% of maternal meiotic errors are found to occur during meiosis I (MI). Originally, the remaining errors were thought to occur during meiosis II (MII), as the pericentromeric markers were reduced to homozygosity. However, based on recent data showing an association with increased maternal age (12) and an association with specific recombination patterns (summarized below), we have concluded that so-called MII cases (referred to as 'MII' cases) are the result of an error initiated in MI and continued in MII. Thus, virtually all maternal meiotic errors of chromosome 21 are thought to be initiated in MI.

Molecular markers spanning the length of the long arm of chromosome 21 have been used to generate recombinational profiles among meioses with non-disjunction errors involving chromosome 21 (4,6,14). Recently, these studies were extended to examine the underlying distribution of exchanges estimated from the observed recombination profiles (15). Three exchange configurations were found to increase the risk for chromosome

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21 non-disjunction. First, a large proportion (~45%) of chromosome 21 tetrads involved in MI non-disjunction are estimated to be achiasmate. This finding sharply contrasted the data for normal female meiosis where the frequency of achiasmate tetrads was estimated to be zero (15). These achiasmate tetrads might represent chromosome 21 homologs that failed to pair during prophase I or those that did pair, but failed to engage in genetic exchange.

When there was a recombination event between the chromosomes 21, the distributions of exchange locations estimated from observed recombination profiles among both MI and 'MII' errors were different from those of normal female meiosis. The MI cases were found to have twice as much exchange occurring in the telomeric third of the chromosome compared with controls, whereas the 'MII' cases had nearly twice as much proximal exchange. These data suggested that the proximity of an exchange relative to the centromere established the susceptibility for a tetrad to non-disjoin: exchanges too near the centromere or telomere did not appear to impart the same stability as a more medially located chiasma.

The same altered recombination patterns/exchange distributions were observed along the non-disjoined chromosomes 21 among DS probands born to both younger and older women (6,15). Thus, to account for the maternal age effect and to explain susceptible exchange configurations associated with non-disjunction of chromosome 21, a two-hit model was hypothesized (6). The first hit is unrelated to maternal age and involves the formation of a susceptible tetrad resulting from a specific exchange pattern established prenatally during MI. The second hit involves some age-related disturbance of the meiotic process. Such a disturbance might involve any part of the meiotic apparatus (e.g. gradual deterioration of the resting oocyte, reduced pH or oxygen affecting the spindle, degraded sister chromatid cohesion proteins or chiasma-binding proteins), DNA repair enzymes or environmental exposures (e.g. smoking). Thus, under normal circumstances, homologs with at least one exchange should segregate correctly, irrespective of the position of the exchange event. However, if meiosis is perturbed in some way, the exchange configurations described above may be more likely to undergo improper segregation and non-disjunction. As a woman ages, her chance of a meiotic disturbance increases.

If no exchange occurs between homologs, the risk for non-disjunction during MI is high. Thus, there does not appear to be a back-up system in humans to ensure segregation of achiasmate homologs, although data are too limited to conclude this definitively (7,10). Intuitively, we would expect that maternal age would not be associated with such cases, as there would be no need for a 'second hit'. In fact, we see as many non-disjoined cases of chromosomes 21 with no observable recombinants among young mothers as older mothers (6,15). This may be due to the fact that a large proportion of the 'zero' observable recombinant cases includes those with one or more exchanges that cannot be separated from true achiasmate cases (see Materials and Methods). A larger number of cases are needed to detect such differences in maternal age, if they do exist, among the various recombination profiles.

At least one important question remains with respect to the extent of the altered recombination patterns observed along non-disjoined human chromosomes. Are these patterns confined to the non-disjoined chromosomes, or do they extend to the total genome? If patterns such as reduced recombination do extend to the entire genome, are they drawn from the normal distribution of recombination in females or do they represent some deviation from the normal distribution? If the variant recombination patterns are limited to the non-disjoined chromosome, chromosome-specific risk factors would be implicated. If the altered recombination patterns are cell wide and appear to be drawn from the lower or upper tails of the distribution, a multifactorial threshold model could be envisaged and used as a framework for further studies. Lastly, if the altered recombination is cell wide, but clearly deviates from the normal distribution of recombination, a search for specific mutations involved in the regulation of recombination would be warranted. As both genes and environmental factors are known to play a role in this complex system, narrowing the scope of putative factors is an important step in continuing studies on recombination-associated non-disjunction of human chromosomes.

In the present study, we have focused these questions further: we have asked whether there is reduced recombination in the total genome of an egg with a non-disjoined chromosome 21 and no detectable recombination. We chose this class of non-disjoined chromosomes to enrich for extremes in recombination and, potentially, to minimize variation due to the 'second hit'. Using 366 markers spanning the human genome, we assayed for cell-wide disturbances in both the amount and distribution of recombination events. We report preliminary evidence of a cell-wide reduction in recombination rate in the genome of eggs with non-disjoined chromosomes 21 with no detectable chromosome 21 recombination events. This reduction appears to be global in nature, with no specific chromosomes driving the effect. Furthermore, this reduction seems to be part of the continuum of normal variation of recombination among eggs and not due to aberrant recombination.

RESULTS

Study population

All probands with free trisomy 21 resulted from a maternal MI error with no observed recombination events along the non-disjoined chromosome 21. The total number of participating families was 19, for a total of 95 individuals (proband, father, mother and mother's parents). Maternal ages at the time of the proband's delivery ranged from 24 to 41 years, with a mean maternal age of 33 ± 5 years. No correlation between maternal age and the overall number of detectable recombination events was observed. Seventeen case mothers were Caucasian, one mother was African-American and one mother was Asian-American.

The control sample consisted of normal female meiotic events obtained from eight CEPH families. All individuals were Caucasians. Although the maternal ages of these events were not available to us, previous studies have found no association between the amount of recombination and maternal age in these families (16).

Classification of probands by somatic cell hybrids

Factors involved in the non-disjunction of tetrads with no exchange may be different from tetrads with exchanges. Thus, the uniformity of the case etiologies was maximized using somatic cell hybrid techniques to identify previously 'hidden'

Table 1. Comparisons of the total observed number of genome-wide recombination events

Sample	No. of meioses	Expected no. of chromosome 21 exchanges	Total recombination events (mean ± SD) in:		
			Genome	Centromeres	Telomeres
Cases	15	0.19	35.45 ± 6.30	6.17 ± 2.47	7.90 ± 2.68
Controls					
0 recombinants[a]	41	1.07	38.13 ± 6.26	7.13 ± 2.46	8.18 ± 2.82
≥1 recombinants[a]	50	1.27	41.37 ± 6.96	8.20 ± 2.42	9.06 ± 2.70
All	91	–	39.91 ± 6.81	7.72 ± 2.49	8.67 ± 2.77
Comparisons (*P* value)					
Cases versus controls[b]			0.030	0.063	0.248
Cases, controls with 0 and ≥1 rec.[c]		0.007	0.014	0.188	

[a]Controls grouped by number of observed recombinants on chromosome 21.
[b]Mann–Whitney *t*-test, two-sided *P* values between cases and all controls.
[c]Kruskal–Wallis test, two-sided *P* values comparing three groups: cases, controls with 0 and ≥1 observed recombinants.

exchanges along the non-disjoined chromosomes 21 (see Materials and Methods). Of the 19 probands, four were excluded for the following reasons. For one proband, the prerequisite lymphoblastoid cell line transformation was done three times but the prolific cell growth required for fusion could not be achieved. For another proband, hybrids were established, but characterization gave inconclusive results: three hybrids indicated chromosomes with the maternal phase intact, whereas three other hybrids suggested a hidden single exchange. This case perhaps resulted from breakage and rearrangements during fusion. Among the remaining characterized 17 cases, one hidden single exchange and one hidden double exchange were found, and these were also excluded. Thus, for the 15 remaining probands, the only type of exchange tetrad that could not be detected was one in which both non-exchange chromatids segregated into the egg. The probability of this occurrence was estimated to be 19% (see Materials and Methods). These 15 cases will be referred to as 'non-exchange' tetrads for the sake of brevity.

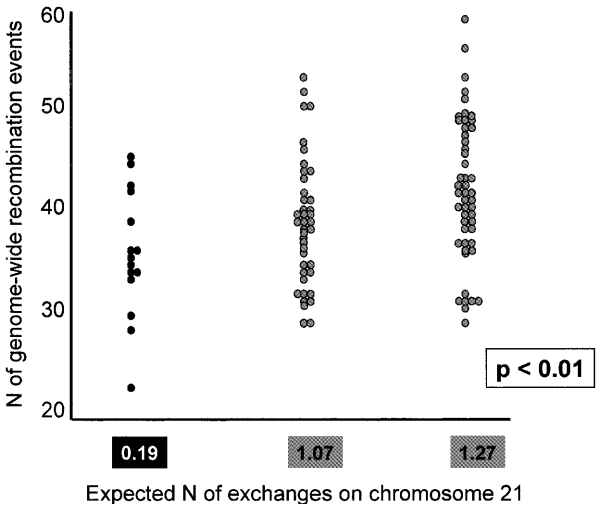

Figure 1. Scatter plot illustrating the total recombination events among the cases (black circles) and controls (gray circles) grouped by the mean of the expected number of exchanges along chromosome 21.

Comparison of genome-wide recombination events

Our primary interest was to determine whether the reduction in recombination observed along the non-disjoined chromosomes 21 among maternal MI errors was limited to the non-disjoined chromosome or if it was part of a cell-wide effect. We found a statistically significant reduction in the total number of recombination events occurring throughout the genome of the 15 cases (35.5 ± 6.3) compared with the 91 controls (39.9 ± 6.8) ($P < 0.05$) (Table 1). Although not statistically significant, both the centromeric and telomeric regions of chromosomes seemed to show the same pattern of reduction. It is interesting to point out that two of the case probands had lower amounts of overall recombination (22.4 and 26.9) than any of the 91 control individuals, where 28.0 was the lowest observed total (Fig. 1).

If these differences were due to an overall reduction of recombination in the gamete, all chromosomes should show this reduction. Thus, we compared the total recombination events along each chromosome for cases and controls (Table 2). Sixteen chromosomes had slightly less recombination in cases than in controls, and the remaining six chromosomes had slightly more (Table 2). None of the differences were dramatic in size and none were statistically significant after correcting for multiple comparisons.

This overall reduction in recombination between cases and controls may be due to normal genetic variation in recombination which was revealed because we selected a subset of cases with no recombination along their non-disjoined chromosome 21 and compared them with a control group unselected for recombination. Thus, our case and control chromosomes 21 differed because of the non-disjunction status as well as the recombination status. Unfortunately, it is impossible to separate these two factors, as the ideal control group cannot be obtained: comparable control gametes with non-exchange chromosome 21 tetrads that disjoined properly are rare, if they exist at all (15), and cannot be identified readily. However, we could separate controls into two groups based on the absence or presence of observable chromosome 21 recombination to determine whether there was a continuum with respect to recombination among groups defined by their expected number of exchanges per chromosome 21. As discussed in Materials and Methods, the expected number of

Table 2. Chromosome-specific comparisons of observed number of recombination events

Chromosome	Mean no. of recombinants per chromosome				Comparisons	
	Cases	Controls			Cases versus controls (P value)[b]	Pattern among cases, 0 and ≥1 rec. controls[c]
		0 rec.[a]	≥1 rec.[a]	All		
1	3.01	3.15	3.31	3.24	0.45	+
2	2.79	3.27	2.96	3.10	0.98	0
3	2.34	2.43	2.72	2.59	0.84	+
4	1.74	2.36	2.37	2.36	0.02	+
5	2.50	2.43	2.31	2.36	0.77	–
6	2.12	2.12	2.38	2.26	0.60	0
7	1.80	2.03	2.44	2.26	0.36	+
8	1.58	2.20	1.96	2.07	0.02	0
9	1.41	1.61	1.88	1.76	0.30	+
10	1.76	1.70	2.18	1.97	0.22	0
11	1.69	1.36	1.41	1.39	0.32	0
12	1.57	1.73	2.02	1.89	0.41	+
13	1.18	0.95	1.33	1.15	0.84	0
14	1.01	1.23	1.38	1.31	0.51	+
15	1.37	1.16	1.30	1.24	0.62	0
16	1.07	1.48	1.51	1.50	0.06	+
17	1.34	1.48	1.47	1.48	0.10	0
18	1.15	1.45	1.54	1.50	0.19	+
19	1.22	0.94	1.05	1.00	0.53	0
20	1.03	0.85	1.32	1.11	0.17	0
22	0.35	0.44	0.68	0.57	0.06	+
X	1.94	1.77	1.83	1.80	0.76	0

[a]Controls grouped by number of observed recombinants on chromosome 21.
[b]Mann–Whitney t-test, two-sided P values between cases and all controls; no correction for multiple tests.
[c]This is a simple non-statistical comparison of increasing (+), decreasing (–) or no (0) pattern among the means of the cases, controls with no recombination and those with ≥1 recombinants.

exchanges per chromosome 21 for case probands, on average, would be 0.19. The expected number of exchanges per chromosome 21 for controls with no observable recombination (n = 41) was estimated to be 1.07 and for controls with recombination (n = 43 for one recombinant and n = 7 for two recombinants), 1.27. These estimations were done using the method of Lamb *et al.* (7) and the comprehensive set of chromosome 21 markers (http//www.marshmed.org/genetics).

A statistically significant difference among these three groups was identified ($P < 0.01$) using a non-parametric analysis of variance (Table 1). This difference did not appear to be limited to specific regions of the chromosome, as the recombination in the centromeric and telomeric regions showed the same increasing recombination pattern with increasing expected exchanges per chromosome 21 (although not statistically significant for the telomeric region). Although we did not perform statistical tests to compare chromosome-specific recombination patterns due to the low power in the sample, the association seemed to be global, i.e. the same increasing number of recombination events with increasing expected number of chromosome 21 exchanges was observed among the three groups for many of the chromosomes: 10 chromosomes showed an increasing amount of recombination with increasing expected exchanges per chromosome 21, one showed a decreasing pattern and the remaining 11 showed no simple pattern among the three groups.

DISCUSSION

There is a well-established association between altered recombination and non-disjunction of human chromosomes. Three classes of chiasmate configurations seem to be more susceptible to non-disjunction than others: (i) non-exchange, or achiasmate, tetrads; (ii) tetrads with a distally placed exchange; and (iii) tetrads with a pericentromeric exchange. These susceptible classes are observed in humans and in *Drosophila* (for a review see ref. 17).

To date, all studies of non-disjoined chromosomes have examined the behavior of recombination only along the non-disjoined chromosome. Thus, it is not known whether there is a genome-wide effect on recombination in an egg with a non-disjoined chromosome or whether altered recombination is specific to the non-disjoined chromosome. In this report, we present the results from a genome-wide recombination screen assaying for such putative cell-wide patterns in a human egg including non-disjoined chromosomes 21 with one of the three susceptible configurations, namely a non-exchange chromosome 21 tetrad. We found the first, albeit preliminary, evidence of a global reduction in total observed recombination events

occurring in such eggs. This reduction did not seem to be driven by particular regions of chromosomes (i.e. centromeric or telomeric) or by particular chromosomes, rather it appeared to be cell wide; however, the power to detect such variation was low.

We cannot state conclusively that this reduction in genome-wide recombination is a specific risk factor for non-disjunction of chromosome 21 because of our study design. We selected our case probands based on two characteristics of their chromosomes 21: non-disjunction and no detectable chromosome 21 exchanges. In contrast, we selected our controls on only one of those characteristics: the chromosomes 21 had undergone normal segregation. Thus, the observed reduced recombination in the case genomes may be associated only with the recombination profile of chromosome 21. To examine this, we grouped the controls by the absence or presence of observed recombination and, indeed, found a difference in the mean number of cell-wide recombination events among these groups, i.e. when we redefined our case–control sample into three groups based on the expected number of exchanges per chromosome 21 (0.19, 1.07 and 1.27 estimated exchanges for cases, controls with no observed recombination events and controls with recombination, respectively), we observed an increase in the mean number of genome-wide recombination events with increasing number of exchanges per chromosome 21.

Although our design limits any direct conclusion with respect to an association of reduction of genome-wide recombination and chromosome 21 non-disjunction, data from model systems support this possibility. For example, many mutations resulting in decreased global recombination or loss of crossover control are accompanied by an increase in non-disjunction at MI (for yeast see ref. 18; for *Drosophila* see refs 19–21; for *Caenorhabditis elegans* see ref. 22). Of particular interest are the studies on a mild recombination-defective mutation *mei-S282* that causes a decreased amount of global recombination in *Drosophila* (23,24). In *mei-S282*$^{-}$ strains, the fraction of zero-exchange tetrads increases whereas those for single- and double-exchange tetrads decrease. Furthermore, only non-exchange tetrads non-disjoin in the presence of this mutation. Thus, these studies are similar to our present study in that the researchers compared the rate of achiasmate X chromosome non-disjunction while examining recombination on one arm of the second chromosome. The frequency of X chromosome non-disjunction was increased significantly among oocytes with non-crossover second chromosomes. Thus, it appears that non-disjunction of the achiasmate X is more frequent when exchange is reduced on other chromosomes. Presumably, if exchange along the entire second chromosome were to be measured among X exceptions, a result similar to those of our present study might be observed.

Additionally, data from model systems suggest that the genetic control of crossover frequency results from both *cis*- (i.e. sequence, position or chromatin structure) and *trans*- (e.g. recombination and repair machinery) acting factors affecting recombination rates over a chromosome segment. Clearly, recombination rates over the entire genome or over a small chromosome segment should be considered as a multifactorial trait and, consequently, include inter-individual differences.

Evidence in humans for significant inter-individual variation in the rate of total genomic recombination among women was shown by Broman *et al.* (16) and was independent of maternal age. The source of the genetic contribution to this variation could arise at several potential points during the process of meiotic recombination: from the initial step of homolog pairing to the final step of resolution into chiasmata (25). Additionally, environmental effects also have an effect on recombination rates. For example, in many organisms, increased temperature or X-rays can increase the number of total chiasmata (for a review see ref. 25).

The apparent cell-wide reduction in recombination observed in the eggs with non-disjoined chromosomes 21 and no detectable recombination appears to reflect the low end of normal variation in total recombination events occurring in the human female. We did not observe any patterns in recombination among these eggs that would indicate aberrant recombination, i.e. although the mean of total recombination was significantly shifted downward, the range of case proband values almost completely overlapped the control values. Also, the mean number of recombination events in the centromeric and telomeric regions showed the same pattern of reduction with decreasing number of chromosome 21 expected exchanges. Lastly, the majority of chromosomes showed reduction in recombination compared with controls; thus, one or a few specific chromosomes did not drive this effect. However, this study was limited in ability to detect disturbances in recombination in telomeres and centromeres (see Materials and Methods) and limited in power to detect chromosomal variation. Thus, confirmation of these data is important.

This variation may have genetic and/or environmental origins. Our data suggest that chromosome 21 may be particularly susceptible to variation resulting from *trans*-acting factors regulating recombination, as a small variation in the expected number of chromosome 21 exchanges seemed to be a good predictor of the statistically significant inter-individual variation in genome-wide recombination rates. These findings need to be confirmed on a larger data set in order to rule out the possibility of statistical fluctuation in recombination rates in this relatively small data set. Once confirmed, an analysis of the sibs of trisomic probands would be one potential approach to determine whether the cell-wide reduced recombination was specific to that egg with the non-disjunction event or whether more than one of the mother's eggs demonstrated similar reductions. The former would suggest some egg-specific factor, potentially environmental in nature. Alternatively, if multiple eggs from that mother had lowered amounts of recombination, some kind of maternal effect would be suggested. This maternal effect might originate from her genotype, the fetal environment in which her eggs developed or an interaction between the two.

Our data support the hypothesis that only this subclass of susceptible chromosome 21 chiasmate configurations, namely non-exchange chromosome 21 tetrads, would have reduced recombination in the overall genome. Those susceptible chromosome 21 exchange configurations should show normal levels of genome-wide recombination, as predicted by the presence of a chromosome 21 exchange. Furthermore, the genomes of non-exchange, non-disjoined chromosomes other than chromosome 21 may or may not show the same reduction in recombination. The pattern may depend on the relative importance of *trans*-acting factors regulating recombination compared with chromosome-dependent *cis*-acting factors.

In summary, we found a cell-wide reduction in the total amount of recombination events in eggs with non-disjoined, non-exchange chromosomes 21. Most importantly, we found

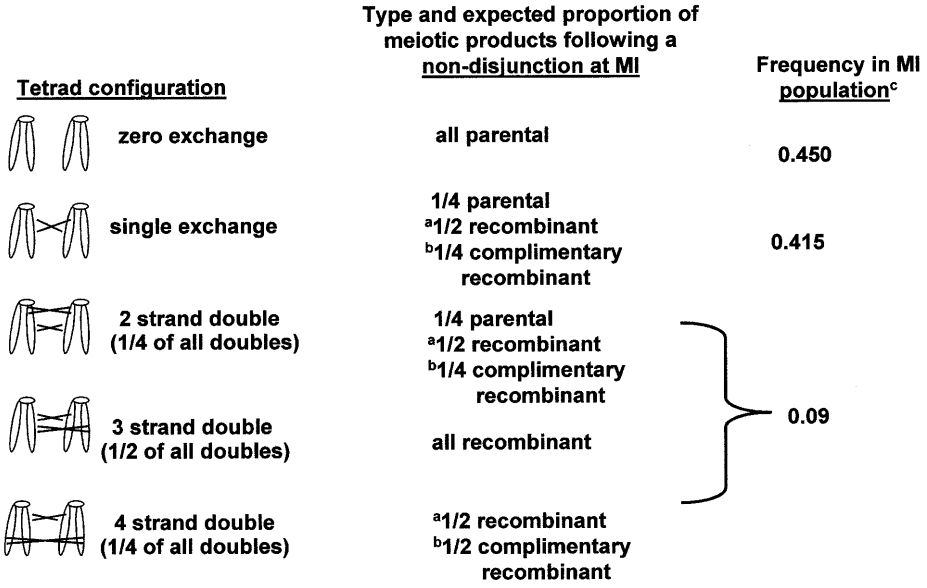

Figure 2. Use of somatic cell hybrids to detect complimentary recombinant strands as adapted from Lander and Green (27). [a]Scenarios detected directly using centromere mapping methods; [b]scenarios detected using SCH; [c]frequencies taken from Lamb *et al.* (26).

that this reduction was part of the normal variation in recombination observed among eggs in general and was predicted by the expected number of exchanges per chromosome 21, irrespective of non-disjunction. This study is preliminary as the number of cases with non-exchange tetrads was small and thus further study is warranted. Studies on other chromosomes report consistent findings of altered, though differing, recombination patterns along non-disjoined chromosomes 15, 16, 18, 21 and the sex chromosomes (17). Thus, in addition to repeating this study on a larger number of trisomy 21 probands, it would be important to determine whether eggs with a non-disjoined chromosome, other than chromosome 21, also demonstrate cell-wide effects. Such studies would be important to test our prediction that non-disjoined chromosomes with exchanges should show no association with total genome-wide recombination rates. Furthermore, such studies may begin to unravel factors that regulate recombination in humans. For example, the ability to predict accurately the level of genome-wide recombination based on the amount of recombination occurring on a specific chromosome may indicate the basis for chromosome-specific regulation of recombination. The next step following confirmation is to determine the factors that affect individual variation in recombination and, more specifically, to determine the extent to which genes, environment and their interactions determine the susceptibility to a non-disjunction error.

MATERIALS AND METHODS

Study population

Cases for this study were drawn from the larger study of live births with free trisomy 21 ascertained from the five county metropolitan area of Atlanta, GA. Blood was obtained from parents and the proband for DNA extraction. Chromosome 21 genetic markers were used to determine the parental and meiotic stage of origin of the non-disjunction error and to examine the recombination pattern along 21q as previously described (4,6,8,11,15). Probands for this study were defined as those with free trisomy 21 due to a maternal MI error with no observed recombination events; the father, mother and both maternal grandparents were required for inclusion in the study. We received blood for DNA extraction from a total of 19 families, with an overall total of 95 individuals.

Inherent misclassification in case definition

Although all of our cases were selected because they were MI errors with no detectable crossovers, we cannot state that each case resulted unambiguously from an achiasmate chromosome 21 tetrad. This ambiguity results from the fact that the observed recombination profiles obtained from the non-disjoined chromosomes only partially represent the exchange patterns that actually occurred between the homologs during MI (15). For example, when an MI non-disjunction occurs that involves a single exchange between the chromosomes 21, there is a 50% chance of not detecting the exchange event: 1 in 4 result from the two complimentary recombinant strands migrating together and 1 in 4 result from the two non-exchange strands migrating together (Fig. 2). We determined the probability that a case proband was misclassified (i.e. their non-disjoined chromosomes 21 were actually derived from an exchange tetrad) by dividing the proportion of 'hidden exchanges' (1 in 2 singles and 1 in 4 doubles) by the proportion of cases that are truly achiasmate plus those 'hidden exchanges'. The estimates of the frequency of achiasmate, single and double exchange tetrads that occur in a maternal MI population are 0.45, 0.415 and 0.09, respectively, and were derived from observed recombinational profiles of cases with maternal MI errors (7). These estimates have large confidence intervals, thus our final probability also has a large confidence interval. Nevertheless, the best estimate for the probability of misclassifying a proband, based on current data, is 0.34. Using a somatic cell hybrid approach for capturing single human chromosomes 21 from trisomy 21 cell lines (26), it

is possible to detect complimentary recombinant chromosomes. By identifying these cases, only 1 in 4 single exchanges and 1 in 16 double exchanges remain as apparent non-recombinants. Thus, we determined that the probability of misclassifying a case proband after the application of somatic cell hybrid techniques was 0.19. This is also the best estimate for the expected number of exchanges per chromosome 21 for this type of non-disjoined chromosomes 21 tetrad.

Somatic cell hybrid fusion protocol

We directly applied the somatic cell hybrid fusion protocol detailed in Shen *et al.* (26). Briefly, lymphoblastoid cell lines from trisomic case probands were expanded to confluency in a T75 flask. These cells were then fused to the CHO purine auxotrophic cell line Ade-C using polyethylene glycol (Boehringer Mannheim, Indianapolis, IN). As Ade-C is deficient for glycinamide ribonucleotide formyltransferase, which is encoded by a gene on human chromosome 21, we were able to select for hybrids retaining human chromosome 21 by growth in hypoxanthine-free medium with 15% serum. Following the fusion, individual colonies were transferred to a 24-well plate, and subsequently passaged, expanded and analyzed.

Characterization of hybrids

DNA was extracted from hybrids using the Puregene kit (Gentra Systems, Minneapolis, MN). The primary difference between the present study and that of Shen *et al.* (26) was our use of maternal grandparents to establish phase of the maternal chromosomes, where Shen *et al.* (26) established hybrids on the mother as well. Thus, our approach was dependent on markers that allowed for phase assignment and were heterozygous in the mother. Hybrids were screened for intact single maternal chromosomes using such markers. Initially, pericentromeric (*D21S369*, *D21S215*, *D21S258*, *D21S192* and *D21S1911*) and telomeric (*COL6A2*, *D21S1261* and *D21S1446*) chromosome 21 markers were used to screen for a single chromosome 21. Further characterization of hybrids appearing intact with only one of the maternal chromosomes 21 was done using medially placed markers (*D21S210*, *D21S214*, *D21S232* and *D21S213*).

Case genome recombination screen

The genome-wide recombination screen among the 19 case families was performed by the Center for Inherited Disease Research (CIDR, Johns Hopkins University, Baltimore, MD) on the DNA extracted directly from blood. CIDR used the Weber screening set version 9.0 replacing 16 markers with similar markers of nearly equivalent heterozygosity. Any markers typed at CIDR that were not included in the screening set or were not serving as replacements for markers in the screening set were removed from the analyses. CIDR's laboratory methods are detailed at their website (http//www.cidr.jhmi.edu).

Control data. The control data set consisted of the eight CEPH families (1331, 1332, 1347, 1362, 1413, 1416, 884 and 102). Although nearly 1 million genotypes are available at the Marshfield website for this control set (http//www.marshmed.org/genetics), only the genotypes for the Weber screening set version 9.0 markers were extracted to ensure that the level of informativeness for the genome-wide recombination screens was equivalent between cases and controls. In keeping with the effort to ensure the same set of markers between the two groups, we removed the following 11 markers from the controls since they were not typed at CIDR: *D1S468*, *D1S3462*, *D5S1462*, *D6S305*, *D7S2195*, *D9S921*, *D11S1984*, *D11S1985*, *D15S818*, *GATA67G11* and *D18S481*. The six chromosome 21 markers were also removed. Finally, the four Y chromosome-specific markers were not included as we were only examining female meiosis. After removal of the above-mentioned markers, the total remaining number of markers included in the present study was 366. The data were screened carefully at Marshfield Genetics for the presence of tight double recombinants and were considered 'clean' (16). Three individuals (1332-09, 1416-10 and 102-05) were removed from our analyses, as they were particularly uninformative having only a few of the screening set markers typed. Thus, after removing these individuals, there were 91 informative female meiotic events among controls. The classification of controls according to the observed recombination on chromosome 21 was done using the full set of chromosome 21 markers, not just the screening set, in order to increase the accuracy of the classification.

Analytical approach

Detection of recombination. The chrompic option of CRI-MAP was used to generate a chromosome picture displaying the grandparental origins of alleles in each child's chromosomes according to the phase with the highest likelihood for that family (27). It gives the number and location of recombination events on each chromosome. For the purpose of this study, we were only interested in the behavior of recombination during female meiosis, so only the maternally inherited chromosomes were studied.

Assigning recombination values for each inter-marker interval. To assay for recombination occurring in specific regions of interest, the distance between two markers was considered as an interval. The crossover value for an interval flanked by alleles of different grandparental origin was equal to 1 (Fig. 3A). To ensure consistent treatment between the case and control data, the following rules were established to handle uninformative markers. If an uninformative marker separated two informative markers showing a crossover event, the crossover was divided between those two intervals. For example, in a three marker string such as '1, –, 0', (where '1' indicates a grandpaternal allele origin, '–' indicates non-informativeness and '0' indicates grandmaternal allele origin), the crossover value for each interval would equal 0.5 (Fig. 3B). Similarly, if more than one non-informative marker separated two markers of different grandparental origin, the crossover value was divided equally among the intervals, as all intervals were of approximately equal genetic size (e.g. two markers separated by three non-informative markers would have four intervals with crossover values equal to 0.25). If non-informative markers separated two markers with the same grandparental origin, the crossover values were defaulted to zero.

To provide a way in which to compare recombination in the centromeric and telomeric regions, ~20 cM intervals were delineated from the marker closest to either the centromere or the telomere. There is no flanking information at some of the

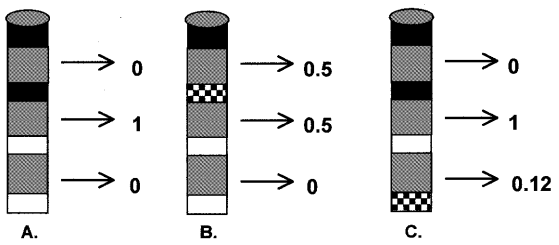

Figure 3. Assigning crossover values to each interval according to grandparental origin of alleles. Gray, intervals; black, allele of grandpaternal origin; white, allele of grandmaternal origin; black and white checkered, allele of unknown origin. (**A**) Fully informative: all markers have alleles with known grandparental origins. (**B**) Non-informative flanked: one marker has an allele with unknown grandparental origin flanked by markers with alleles of different grandparental origin, thus the crossover was equally likely to have occurred in either interval. (**C**) Non-informative non-flanked: the most telomeric marker has an allele of unknown grandparental origin. The female-specific genetic distance between this marker and the next informative marker equals 12 cM, thus the probability that a crossover occurred in the most telomeric interval was set to 0.12.

centromeres (i.e. the acrocentric chromosomes) and the telomeres. If the markers at these non-flanked regions were non-informative, the crossover value between those non-informative markers was set equal to the inter-marker genetic distance. For example, if the female-specific genetic distance between the most telomeric marker and the next marker was 12 cM, and the most telomeric marker was non-informative, the probability of a crossover for that interval was set to 0.12 (Fig. 3C).

Although the coverage of the version 9 Weber marker set is good, coverage of intervals in the centromeric and telomeric regions may be limited. This is due primarily to the fact that for most chromosomes, there is no marker that is located in these specific physical regions and the distance from the closest genetic marker to the physical telomere or centromere is unknown. Also, polymorphisms in these regions are sometimes complex and therefore not included in a screening marker set. Thus, we may not have the power to identify specific disturbances in recombination in the extremes of these regions.

Statistical tests. We used the Mann–Whitney test for all comparisons in order to account for the sample size and because the distributions among the smaller regions of interest (e.g. individual chromosomes, centromeric and telomeric regions) were not distributed normally. All reported significance levels are based on two-sided *P* values. However, for our primary hypothesis, this will be conservative as we were testing specifically for a reduction in the amount of recombination among the case genomes. For the chromosome-specific comparisons, the two-sided *P* values are appropriate as there was no reason to suspect a reduction in recombination across all the chromosomes and it was impossible to predict which individual chromosomes would demonstrate reduced amounts of recombination.

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