Mini Review

Down syndrome: genetic recombination and the origin of the extra chromosome 21


Despite the clinical importance of trisomy 21, we have been ignorant of the causes of meiotic nondisjunction of chromosome 21. Recently, however, genetic mapping studies of trisomy 21 families have led to the identification of the first molecular correlate of human nondisjunction; i.e. altered levels and positioning of meiotic recombinational events. Specifically, increases in 0 exchange events or in distal-only or pericentric exchanges are significantly increased in trisomy 21-generating meioses. These observations have led to the idea that chromosome 21 nondisjunction requires ‘two hits’: first, the establishment in prophase I of a ‘vulnerable’ bivalent and second, abnormal processing of the bivalent at metaphase I or II.

Trisomy 21, the chromosome abnormality responsible for over 95% of Down syndrome (DS), is the most important genetic cause of mental retardation in humans, occurring in approximately 1 in 600–800 live births (1). Furthermore, these individuals represent a relatively small proportion of all trisomy 21 conceptions; over 80% of such conceptsuses perish in utero and these account for approximately 1–2% of recognized spontaneous abortions (2). Thus, trisomy 21 is an important cause of human pregnancy failure, as well as a leading contributor to mental retardation.

Although the chromosomal basis of DS has been known for 40 years (3), there is still a surprising lack of knowledge about the causes of nondisjunction and about factors that may predispose to trisomy 21. However, recent molecular and epidemiological studies have begun to shed light on the origin and etiology of trisomy 21. In this review, we summarize recent data on the origin of trisomy 21, with an emphasis on the roles of aberrant recombination and maternal age in the genesis of the additional chromosome 21.

The origin of trisomy 21

Over the past several years, DNA polymorphisms have been used to investigate the parent and meiotic stage of origin of approximately 2000 trisomic fetuses and liveborns. Trisomy 21 has been the most extensively studied condition, with over 1000 informative cases now being reported. Results of analyses conducted in our laboratories on free, apparently nonmosaic, trisomy 21 cases are summarized in Table 1. The results of these studies indicate that the vast majority of errors leading to trisomy 21 are due to errors in the egg, as nearly 90% of cases involve an additional maternal chromosome. Approximately 10% result from paternal meiotic nondisjunction and a small proportion (1.8%) are attributable to post-zygotic mitotic nondisjunction. From Table 1, it is also clear that the timing of meiotic nondisjunctional errors differs among egg and sperm: the ratio of errors scored as meiosis I (MI) compared with meiosis II (MII) in eggs is 3:1, whereas in sperm is 1:1. Thus, the frequency and perhaps the mechanism of nondisjunction depend on the gametic context in which the chromosome resides.

Table 1 also provides information on mean maternal age by the mechanism of origin of trisomy. While comparable information on controls is not available for the entire data set, these results are nevertheless instructive – they indicate that the maternal age effect is restricted to cases of maternal origin, and that cases of trisomy 21 of paternal
Hassold and Sherman

Table 1. Summary of the parental origin and timing of the nondisjunctional error for chromosome 21 ([4] and S. Sherman, unpublished data)

<table>
<thead>
<tr>
<th>Parental origin</th>
<th>Timing of error</th>
<th>No. of cases</th>
<th>Origin (%)</th>
<th>Proportion among origin (%)</th>
<th>Mean maternal age ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal</td>
<td>MI</td>
<td>453</td>
<td>75.4</td>
<td>31.3 ± 0.3</td>
<td>31.5 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>‘MII’</td>
<td>148</td>
<td>24.6</td>
<td>32.1 ± 0.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Unknown</td>
<td>46</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Paternal</td>
<td>MI</td>
<td>26</td>
<td>45.6</td>
<td>27.4 ± 1.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>‘MII’</td>
<td>31</td>
<td>54.4</td>
<td>27.5 ± 1.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Unknown</td>
<td>7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mitotic</td>
<td></td>
<td>13</td>
<td>1.8</td>
<td>27.4 ± 1.3</td>
<td></td>
</tr>
</tbody>
</table>

or mitotic origin are age independent. Furthermore, among maternally derived cases, there is no difference in the means of MI and MII cases, suggesting that both types of errors are maternal age dependent. This interpretation has been confirmed in a separate population-based study of trisomy 21, in which it was possible to generate maternal age-specific birth prevalence rates for both maternal MI- and MII-derived cases of trisomy 21 ([5]).

Genetic recombination and nondisjunction of chromosome 21

Despite years of intensive study, we still know relatively little about factors that influence the frequency of trisomy 21 in humans. However, recent molecular studies have identified alterations in genetic recombination as an important predisposing factor in both age-independent and age-dependent trisomy 21.

Recombination and maternal nondisjunction

In lower organisms, mutants that reduce meiotic recombination invariably have increased frequencies of nondisjunction (e.g., [6]). In humans, Warren et al. ([7]) provided the first evidence of an association between aberrant recombination and trisomy when they reported reduced levels of chromosome 21 recombination in meioses leading to trisomy 21. Subsequently, several laboratories have extended these observations, and it is now clear that most – if not all – human trisomies are associated with alterations in recombination. For example, significant reductions in recombination have been reported for paternally and maternally derived sex chromosome trisomies and for maternal trisomies 15, 16, 18, and 21 ([8–12]). However, as most of the studies have involved relatively small data sets, there has been little opportunity to characterize the recombination effects, other than in a very general sense.

Because of its relatively high incidence among liveborn individuals, trisomy 21 provides an opportunity to conduct detailed analyses of the association of recombination and nondisjunction for a specific chromosome. In our laboratories, we have used centromere-gene mapping methods ([4]) or analyses of meiotic exchange configurations ([13]) to study patterns of meiotic recombination in normal meioses and in meioses leading to trisomy 21. Our results confirm the association of altered recombination and nondisjunction and indicate that the relationship is a complex one. Specifically, there appear to be at least three different recombination-associated ‘routes’ to maternal chromosome 21 nondisjunction (Fig. 1). First, nearly one-half of maternal MI-derived trisomies involve meioses in which there is an outright failure of recombination between the homologous chromosomes 21 ([13]). Such ‘achiasmate’ bivalents rarely, if ever, occur in normal female meioses ([13]), indicating that failure to cross-over imparts an extraordinary risk of MI mal-segregation to the homologous chromosomes. This is not surprising, as chiasmata are known to be responsible for holding meiotic homologues together in prophase of MI; indeed, studies of meiotic mutants in lower organisms indicate that absence of meiotic exchange is a common source of aneuploidy.

![Fig. 1. Different types of ‘susceptible’ meiotic cross-over configurations. Risk values are based on the relative incidence of each of the different types of configurations in normal- and trisomy 21-generating meioses, as estimated in reference (13).](image-url)
Chiasmata are frequently thought to be both necessary and sufficient for proper meiotic segregation. Thus, the other two recombination-associated routes to maternal trisomy 21 are more surprising, as each involves bivalents in which at least one exchange has occurred (i.e., 'chiasmate' bivalents). In one route, observed in the majority of all chiasmate maternal MI nondisjunctional events, a single exchange is observed in distal 21q. While such situations are also observed in normal female meioses, they are significantly more common in the nondisjunctive meioses. Thus, it appears that 'distal-only' exchanges are less efficient at segregating chromosomes than are medially placed exchanges. Possibly, in the absence of a more proximally located chiasma, the bivalent is less likely to orient the kinetochores to the opposing poles.

Unexpectedly, as recombination occurs at MI, the final route involves maternal MII errors. However, in this instance, the effect involves increased recombination in proximal 21q (11). That is, pericentromeric exchanges are more common in meioses associated with maternal MII trisomy 21 than in normal female meioses. Possibly, proximal chiasmata predispose to 'chromosome entanglement' at MI, with the bivalent passing intact to the MII metaphase plate; assuming that it divides reductionally at MII, the result would be a disomic gamete with identical centromeres, leading to it being scored as an MII-derived trisomy. Alternatively, the presence of a proximal chiasma may interfere with normal sister chromatid cohesion, leading to a premature separation of sisters at MI; if the sister chromatids migrate to the same pole at MI, they would have a 50% chance of traveling together at MII, resulting in an apparent MII nondisjunction.

Taken together, the data on recombination and nondisjunction suggest that, at least for chromosome 21, the susceptibility for a bivalent to nondisjoin is associated with the distance between the centromere and the nearest exchange. This result challenges the widely held concept that events occurring at MI are independent of events occurring at MI, and suggests that most, if not all, maternal nondisjunction is initiated during MI and simply resolved at either of the two meiotic stages. Accordingly, we now refer to nondisjunction at maternal MII as 'MII' events, to indicate that these trisomies likely originate at the first meiotic division.

Further, these data suggest that the chromosome 21 nondisjunction may require at least two events, or 'hits' (4). The first hit would involve the establishment of a susceptible meiotic configuration (i.e., a bivalent with no exchanges or with an exchange at an 'unfortunate' location). As recombination occurs prenatally in the human female, this event would occur in the fetal ovary. The second hit would involve degradation of a meiotic process (e.g., a spindle component, a sister chromatid cohesion protein, a meiotic motor protein, a checkpoint control protein) that increases the risk of improper segregation for these susceptible bivalents. Under normal meiotic conditions, the presence of a single chiasma -- regardless of its location -- may be sufficient for proper chromosome segregation. However, when there is a disturbance of meiosis as a result of maternal age and/or environmental exposures, specific configurations would be more susceptible to nondisjunction than others.

While direct evidence for such a process is lacking in the human, or indeed in any mammalian species, ample support is available from other organisms. For example, in Drosophila females, a number of meiotic mutations that cause nondisjunction of nonexchange chromosomes (e.g., nod, Axs, Dub, and ncd) also allow nondisjunction of exchange bivalents, virtually all of which exhibit distal cross-overs (14–16). As the molecular components of meiosis are extraordinarily conserved throughout evolution, it seems reasonable to assume that similar processes are at work in human meiosis.

Recombination and paternal nondisjunction

Because most trisomy 21 is maternal in origin, relatively little is yet known about the association of altered recombination and paternal chromosome 21 nondisjunction. However, at least some of the relationships observed for maternal trisomy 21 appear to apply to paternal errors as well. For example, among paternal MI errors, a large number are estimated to be achiasmate (17). Possibly, this susceptibility is related to the fact that normally only one chiasma is formed on male chromosomes 21, similar to the susceptibility proposed for the XY bivalent (8).

Maternal age, recombination, and trisomy 21

The association between increasing maternal age and trisomy is arguably the most important etiological factor in human genetic disease. Among women under the age of 25 years, approximately 2% of all clinically recognized pregnancies are trisomic, but by the age of 36 years this value increases to 10%, and by the age of 42 years to over 33% (18). The influence of age is exerted without respect to race, geography, or socioeco-
nomic factors and likely affects segregation of all chromosomes. The relationship between maternal age and trisomy 21 is especially intriguing – like most trisomies, it displays a sharp increase in incidence in the mid-thirties, with the estimated frequency in clinically recognized pregnancies climbing to nearly 5% at age 42 years. However, there also appears to be an 'inverse' age effect, as population-based studies of trisomy 21 have typically observed an increase in incidence at the youngest maternal ages (e.g., (19, 20)).

Despite the importance of increasing age, we know almost nothing about the basis of the effect. It is thought to originate in maternal MI, because in human females, oocytes enter meiosis during the fetal period and remain arrested in prophase of MI until ovulation. As a result, completion of the first meiotic division may take 40 years or longer. The duration of the division suggests that any of four time periods may be important in maternal age-dependent nondisjunction. These include 1) the premeiotic stage when rapid mitotic divisions occur, 2) early MI when pairing and recombination occur, 3) the arrested or dictyate stage, and 4) the peri-ovulatory stage when meiosis resumes and proceeds to the metaphase of MII.

Current data do not allow us to distinguish between factors acting at the four different stages of oogenesis. However, recent data indicate that alterations in genetic recombination are an important contributor to age-dependent nondisjunction of chromosome 21. Specifically, we have observed that, by comparison with normal female meiotic maps, the chromosome 21 genetic maps associated with maternal MI errors are similarly reduced in younger and older women, and the maps associated with so-called 'MII' errors are similarly increased in younger and older women (4). The relationship between maternal age, recombination, and chromosome 21 nondisjunction is also apparent from Table 2. This shows the number of observed recombination events in nondisjunctional meioses by the age of women for both MI events and for events scored as arising at MII. From this it appears that, for MI trisomies, the number of meioses with 0, 1, and 2 recombinants is unaffected by maternal age; similarly, age does not appear to influence the number of meioses with 1 and 2 recombinants in the 'MII' trisomies.

We interpret these data as being consistent with a two-hit model of maternal age-related nondisjunction. That is, the first hit, which is age independent, would involve the establishment of a ‘vulnerable’ chiasmate configuration (e.g., a bivalent with a single, distally placed exchange) in the fetal oocyte; the second hit, which is age dependent, would involve abnormal processing of the vulnerable bivalent at metaphase I. If correct, this interpretation means that the chromosome 21 nondisjunctional process is the same in younger and older women; it simply happens more frequently with age, possibly due to age-dependent degradation of meiosis-specific events.

### Environmental risk factors in trisomy 21

Advanced maternal age and altered recombination remain the only risk factors conclusively associated with nondisjunction in humans. Cytogenetic and epidemiological studies have identified many candidates for extrinsic risk factors, including smoking, alcohol, maternal irradiation, fertility drugs, oral contraceptives, and spermicides. However, unequivocal proof is still lacking for these and other intrinsic and extrinsic factors.

There are several possible explanations for the failure to conclusively identify these factors. It may be that the appropriate factors have not been studied or, possibly, that the ability to identify such factors is limited by study design. A major concern...
in most previous studies is that the etiology of nondisjunction has been considered to be homogeneous, whereas we know that there are different types of meiotic and mitotic errors (Table 1), with the underlying mechanisms being presumably quite different for each. Thus, we instituted an epidemiological study of trisomy 21, utilizing cases in which the parent and timing of nondisjunction are known. Our initial results on approximately 250 trisomy 21 liveborns and a similar number of control liveborns have been instructive (21). To date, we have been focusing on a small number of potential etiological agents, with the results on smoking being the most intriguing. When all maternally derived trisomies are combined for analysis, we find no significant association between maternal smoking at the time of conception and the risk of nondisjunction of chromosome 21. However, when cases are categorized by stage of origin (MI or ‘MII’) and maternal age (<35 or ≥35 years), we observe a significant association between smoking and nondisjunction in the younger women, with the effect being confined to ‘MII’ cases. Furthermore, in an examination of the possible interaction of smoking and oral contraceptive use around the time of conception, we observe a significantly increased odds ratio over that for smoking alone among ‘MII’ cases involving younger mothers. These results are clearly preliminary and needed to be confirmed on a more extensive series of cases. Nevertheless, they provide optimism that, by categorizing trisomies by the mechanism of origin of the extra chromosome, it finally may be possible to identify agents that contribute to human trisomy.

Summary

Studies of the past decade have provided valuable information on the relative contribution of paternal and maternal nondisjunction to trisomy 21. It is now clear that the vast majority of DS is maternally derived, with paternal and mitotic errors playing relatively minor roles. Furthermore, genetic linkage analysis has identified the first molecular correlate of trisomy 21, namely alterations in genetic recombination, and has provided evidence that most maternally derived cases of DS – even those scored as arising at MII – have their origins at MI.

However, the underlying molecular causes of trisomy 21 remain elusive, and the importance of extrinsic or intrinsic risk factors to nondisjunction is still unclear. A combination of approaches – e.g., development of mammalian models of human nondisjunction, continued epidemiological studies of human trisomies, and identification of loci important in mediating mammalian meiotic chromosome pairing, recombination, and segregation – will be necessary before we are able to understand the causes for the errors responsible for trisomy 21 and other human aneuploid conditions.

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References