

# Risk factors for nondisjunction of trisomy 21

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**Abstract.** The leading cause of Down syndrome (DS) is nondisjunction of chromosome 21 occurring during the formation of gametes. In this review, we discuss the progress made to identify risk factors associated with this type of chromosome error occurring in oogenesis and spermatogenesis. For errors occurring in oocytes, the primary risk factors are maternal age and altered recombination. We review the current progress made with respect to these factors and briefly outline the potential environmental and genetic influences that may play a role. Although the studies of paternal nondisjunction are limited due

to the relatively small proportion of errors of this type, we review the potential influence of paternal age, recombination and other environmental and genetic factors on susceptibility. Although progress has been made to understand the mechanisms and risk factors that underlie nondisjunction, considerably more research needs to be conducted to dissect this multifactorial trait, one that has a considerable impact on our species.

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In 1866, John Langdon Down published his classic article that described individuals with a common phenotype that eventually came to bear his name (Down, 1866). The hallmarks of Down syndrome include mental retardation, hypotonia, and abnormalities of the face, hands, and feet. Other variable components of the phenotype include congenital heart defects, digestive tract abnormalities, congenital cataracts and leukemia. With the advent of cytogenetic karyotyping, the etiology of DS was identified in 1959 as the presence of an extra chromosome 21 (Book et al., 1959; Ford et al., 1959; Jacobs et al., 1959; Lejeune, 1959). Trisomy 21 is now one of the most intensively studied human aneuploid conditions. It is one of the few autosomal trisomies that survive to term, although 80% of conceptions with trisomy 21 are spontaneously aborted (Hook et al.,

1995). The incidence of DS, approximately 1 in 600 to 1 in 1000 live births, makes this syndrome the most commonly identified form of mental retardation and a leading cause of birth defects.

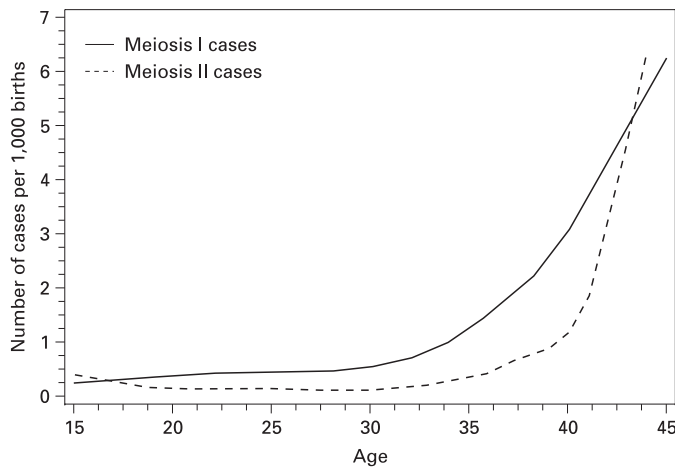
Of individuals with DS, 95% have an extra chromosome 21 as a result of a meiotic nondisjunction error in the segregation of chromosomes 21 during the formation of gametes. Of the remaining 5%, less than 1% is due to somatic mosaicism and the rest to translocations involving chromosome 21. In this review, we will describe risk factors associated with meiotic nondisjunction of chromosome 21, this being the overwhelming cause of trisomy 21. Several extensive studies of possible risk factors have been conducted, however most have not included cytogenetic and/or DNA studies to characterize the type of error leading to trisomy 21 (e.g., Stoll et al., 1998; Carothers et al., 2001; Jyothy et al., 2001; Fisch et al., 2003). In this review, where possible, we will focus on studies that have combined cytogenetic and molecular techniques to group meiotic nondisjunction errors by the parent in whom the error occurred (maternal or paternal error reflecting an error occurring during formation of egg or sperm, respectively) and by the timing of the error during either meiosis I (MI) or meiosis II (MII).

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**Table 1.** Origin of chromosome 21 nondisjunction error and mean maternal age. Unpublished data from the Atlanta Down Syndrome Project, 1989–2002

Origin	Meiotic error	Number of cases	Percent of error type	Mean $\pm$ s.d. maternal age
Meiotic – maternal	MI	240	MI/(MI+MII) = 240/311 = 77.2% MII/(MI+MII) = 71/311 = 22.8% maternal/all = 311/348 = 89.4%	30.98 $\pm$ 6.81
	MII	71		31.44 $\pm$ 7.60
Meiotic – paternal	MI	12	PI/(PI+PII) = 12/22 = 54.5% PII/(PI+PII) = 10/22 = 45.5% paternal/all = 22/348 = 6.3%	28.50 $\pm$ 7.51
	MII	10		26.00 $\pm$ 5.39
Mitotic		15	mitotic/all = 15/348 = 4.3%	29.73 $\pm$ 5.06
Controls		493		27.50 $\pm$ 6.11



**Fig. 1.** Maternal age-specific incidence rates for infants with trisomy 21 due to meiosis I and to meiosis II nondisjunction. (Data points are smoothed and are based on number of infants with trisomy 21 divided by the number of all infants born in the same years from the same geographical area grouped by yearly maternal ages.)

### Maternal nondisjunction

The ability to accurately determine the parental origin of the nondisjunction error and the type of error (MI, MII or mitotic) has enhanced the study of risk factors for trisomy 21. Table 1 shows the breakdown of nondisjunction errors taken from a population-based study of infants born in Atlanta, GA, USA, between 1989–2002 (unpublished data). These data are similar to other population-based series (e.g., Mikkelsen et al., 1995; Gomez et al., 2000) and indicate that over 90% of nondisjunction errors leading to trisomy 21 occur in the oocyte and that the majority of those errors occur during the first stage of meiosis. As discussed below, there is evidence that perhaps almost all nondisjunction errors of chromosome 21 in oocytes are initiated during MI.

#### Maternal age

The age of the mother at the time of the conception of a fetus with DS is, by far, the most significant risk factor for meiotic nondisjunction of chromosome 21. As a woman ages, her risk

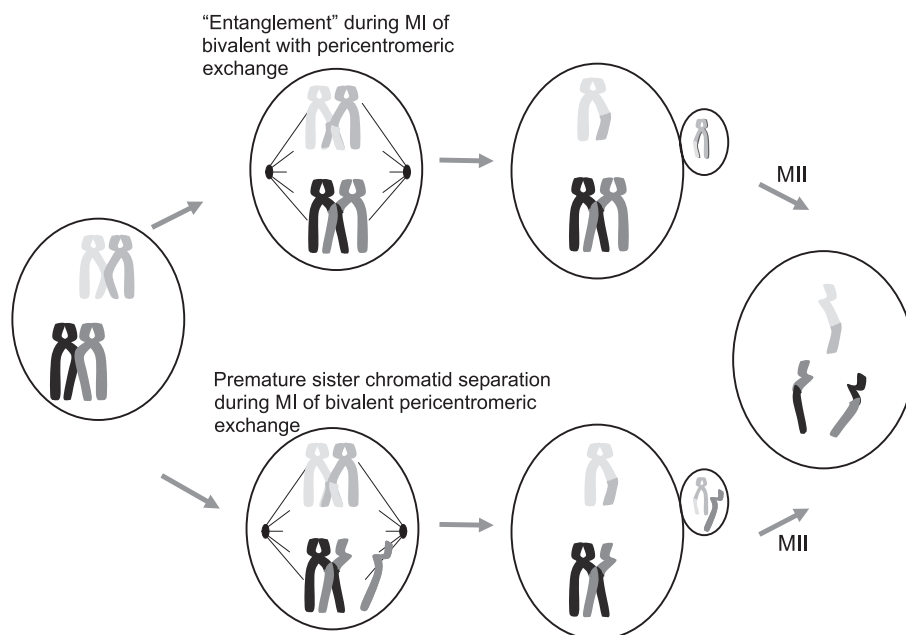
for having a conceptus with trisomy 21 significantly increases. This effect of an increased rate of DS with advancing maternal age was noted by Penrose in 1933 (Penrose, 1933). Although no specific explanation has come to the forefront, significant progress has been made toward characterizing this effect and understanding possible mechanisms.

Table 1 shows the mean maternal age at the time of the birth of a trisomy 21 infant for each type of chromosome error. Taken from a large population-based study of infants (Atlanta Down Syndrome Project, 1989–2002 (unpublished)), these data are similar to others, although the mean maternal ages differ depending on the ascertainment strategy. Most other studies have not been population-based (e.g., Antonarakis et al., 1992; Ballesta et al., 1999; Muller et al., 2000). Nevertheless, important conclusions can be drawn. First, the maternal age effect is restricted to mothers in whom the nondisjunction error occurred. That is, an increased maternal age is not observed among mothers of fetuses who received the extra chromosome 21: 1) through a nondisjunction error in spermatogenesis (paternal errors (e.g., Table 1; Petersen et al., 1993), 2) due to a post-zygotic mitotic error (e.g., Table 1; Antonarakis et al., 1993) and 3) as a translocation (inherited or de novo) (Hook, 1983).

Second, advanced maternal age is a risk factor for both MI and MII maternal nondisjunction errors (e.g., Table 1 which updates data of Yoon et al., 1996; additionally Antonarakis et al., 1992; Muller et al., 2000). This observation potentially ties together the MI and MII errors with respect to risk factors. Interestingly, preliminary data from the Atlanta Down Syndrome Project suggest that the maternal age-specific incidence rates for live births with trisomy 21 may differ between MI and MII nondisjunction errors (Fig. 1): the increasing risk for MII errors is shifted to older maternal ages compared with MI errors, however, this difference is not statistically different. Additional data are needed to confirm this intriguing pattern.

The timeline for oogenesis compared with spermatogenesis points to possible error-prone stages of meiosis. Meiosis is initiated in oocytes during fetal life. After homologous chromosomes synapse and initiate recombination, meiosis is arrested. Meiosis I resumes in the woman's adult life just before the ovulation of an oocyte. At this point, MI is completed and the first polar body is extruded. MII is initiated but goes through a short arrest as it travels down the fallopian tubes. MII is completed after fertilization and the second polar body is extruded. Thus,

**Fig. 2.** Diagram of two possible scenarios for a nondisjunction error that is associated with a pericentromeric exchange: chromosome “entanglement” or premature sister chromatid separation at MI. For each scenario, the resulting disomic gamete has identical centromeres (denoted by the same color); such an error would be scored as originating at MII even though the precipitating event occurred at MI. (The bivalent in the upper half of the oocyte shows normal disjoining chromosomes with a medial exchange. The bivalent in the bottom half of the oocyte shows nondisjoining chromosomes with a pericentromeric exchange. Polar bodies at the end of MII not shown.)



meiosis in a woman extends over a 10- to 50-year period with the oocyte being arrested in MI during most of its “lifetime”. This contrasts with spermatogenesis, which begins at puberty when cells entering meiosis move from one stage to the other without delay.

Thus, maternal age related nondisjunction could be due to: 1) an accumulation of toxic effects of the environment during the arrested state of the oocyte, 2) a degradation of meiotic machinery over time while in the arrested state, leading to a suboptimal resumption of MI and MII, 3) a change in ovarian functioning due to suboptimal hormonal signaling, or 4) degradation of the uterine environment. Most likely, several processes are affected by advanced maternal age and thus more than one of the various hypotheses proposed to explain this effect will be correct. Gaulden (1992) and Eichenlaub-Ritter (1996) provide excellent reviews of the hypotheses proposed to explain the maternal age effect. It is clear that such hypotheses need to focus on both the processes directly affecting oogenesis and the environment in which oocytes are formed in an aging woman.

#### *Maternal recombination*

*Recombination along the nondisjoined chromosome 21.* Aside from maternal age, there is only one other factor that has been shown to be associated with an increased susceptibility of maternal nondisjunction, namely altered recombination patterns. Warren et al. (1987) provided the first evidence to suggest that a proportion of maternal nondisjunction errors were associated with reduced recombination along chromosome 21. Further examination has shown that, in addition to the absence of an exchange along the nondisjoined chromosome 21, the placement of an exchange is an important susceptibility factor for nondisjunction. Altered recombination has also been found associated with nondisjunction events among other chromo-

somes. These data are reviewed in Lamb et al. (2005a). Here, we will provide a summary of the most recent data for trisomy 21 (Lamb et al., 1996, 1997, 2005b).

Briefly, examination of recombination along the maternal nondisjoined chromosome 21 has suggested three susceptibility exchange patterns: 1) no exchange leads to an increased risk of MI errors, 2) a single telomeric exchange leads to an increased risk of MI errors, and 3) a pericentromeric exchange leads to an increased risk of so-called MII errors. These patterns are similar to those observed in model organisms: absent or reduced levels of recombination, along with sub-optimally placed recombinant events, increase the likelihood of nondisjunction (Rasooly et al., 1991; Moore et al., 1994; Sears et al., 1995; Zetka and Rose, 1995; Koehler et al., 1996; Ross et al., 1996; Krawchuk and Wahls, 1999). Exchanges too close to the centromere or single exchanges too close to the telomere seem to confer the most instability.

The association of maternal MII errors with a specific recombination pattern suggests that at least some proportion of MII errors are initiated in MI. Perhaps, the presence of a pericentromeric exchange increases the likelihood of chromosome “entanglement” or premature sister chromatid separation at MI, with the resulting disomic gamete having identical centromeres; such an error would be scored as originating at MII even though the precipitating event occurred at MI (Fig. 2). Thus, at least for chromosome 21, maternal “MII” errors may have their genesis in MI. Instead of changing nomenclature, we will simply designate “MII” to indicate this suggestion and refer to the “type” of meiotic error instead of the “stage” of meiotic error.

The link between recombination and human nondisjunction prompts the obvious question: what is the association between altered recombination and the only other known predisposing factor for trisomy, increasing maternal age? That is,

does either the frequency or the location of exchanges vary with the age of the mother? Previous studies of trisomy 21 failed to identify any association; however, the sample size was relatively small with respect to the amount of variation in recombination along this small chromosome (Lamb et al., 1996). An examination of chromosome 15 nondisjunction provided the first evidence of an age/recombination relationship. Robinson et al. (1998) found that among maternal MI-derived errors, the age of the mother was significantly increased among cases with multiple recombinants compared with those having zero or only one detectable recombinant. From this, the authors suggested that cases with multiple recombinants might be more resistant to nondisjunction because of increased stability of the bivalent over time. Similarly, an analysis of maternal nondisjunction of the X chromosome showed that the mean maternal age of cases with recombination was significantly older than that of cases with no recombination (Thomas et al., 2001). This same pattern was observed for trisomy 18, although the difference was not statistically significant (Thomas et al., 2001).

Recently, Lamb et al. (2005b) updated their original reports using a larger population of 400 maternal MI nondisjoined cases and a more refined genetic analysis. Cases were subdivided into three groups based upon the age of the mother at time of the birth of their offspring with DS: mothers younger than 29 years of age ( $n = 126$ ), mothers from 29 to 34 years of age ( $n = 138$ ), and mothers 35 years of age or older ( $n = 136$ ). Even with this increased sample size, the frequency distributions of the number of exchanges within each age group were not significantly different from each other. For example, 52% of bivalents with no exchange were observed in the youngest group, 29% in the middle-aged group, and 45% in the oldest group. Because the variation in number of exchange events along chromosome 21 is small, more data are needed to determine if there is any association with maternal age and the frequency of exchange along chromosome 21.

Interestingly, patterns differed significantly among age groups with respect to the *location* of the exchanges. The proportion of cases with susceptible exchanges (pericentromeric or single telomeric) was highest among the youngest group of mothers and lowest among the oldest group. In fact, the pattern of exchanges among the oldest age group began to mimic the pattern observed among normally disjoining chromosomes 21. For example, among the youngest age group, nearly 80% of the single exchanges occurred in the most telomeric interval compared with 33 and 14% among the middle and oldest age groups, respectively. These distributions were significantly different from the normally disjoining sample for all three trisomic age groups. However, the level of significance declined with increasing age.

One plausible explanation for these findings suggests that multiple pathways lead to nondisjunction, some age dependent and others age independent. In a young woman, meiotic machinery (spindle function, sister chromatid adhesive proteins, microtubule motor proteins, etc.) functions optimally and, as a result, can correctly segregate all but the most susceptible exchange configurations. For young women then, the greatest risk factor for MI nondisjunction is the presence of a susceptible exchange pattern in the oocyte. As a woman ages,

her meiotic machinery is exposed to an accumulation of environmental and age-related insults, becoming less efficient/more error-prone. Suboptimal exchange patterns still increase susceptibility to nondisjunction, but now even bivalents with correct exchanges are at risk. Over time, the proportion of nondisjunction due to normal exchange configurations increases as age-dependent risk factors exert their influence. As a result, the most prevalent exchange profile of nondisjoined oocytes shifts from susceptible to non-susceptible patterns with age of the oocyte.

If “MII” errors are initiated in MI, exchange patterns among maternal age groups with “MII” errors are predicted to be similar to those observed for MI errors. Preliminary data suggest that this is not the case (Lamb, Feingold, and Sherman; unpublished data). In the limited study sample (about 40 cases in each age group), the amount of recombination significantly decreased with increasing maternal age for “MII” errors ( $P < 0.01$ ). For example, the mean age of women with an “MII” error and one observed recombinant was 32.8 years whereas the mean maternal age of those with two or more recombinants was 28.2 years. Moreover, the proportion of susceptible exchanges increased with age, the opposite pattern to that observed in MI. Whether these results are robust remains to be seen. If these results are confirmed, the apparent difference in MI- and “MII”-maternal age associated exchange patterns may provide clues to the nondisjunction mechanism and associated risk factors.

*Genome-wide recombination in the oocyte with a nondisjoined chromosome 21.* An obvious question related to chromosome 21 recombination is to what extent do the altered patterns extend to the rest of the genome? Brown et al. (2000) asked the more specific question: Is there reduced recombination in the total genome of an oocyte with a chromosome 21 MI error and no detectable recombination? They found a statistically significant genome-wide reduction in the mean recombination rate in such oocytes compared with those with normally disjoined chromosomes 21. More importantly, they found that this reduction was consistent with normal variation in recombination observed among all oocytes. Thus, given that recombination is a multifactorial trait, these data suggest that specific chromosomes may be at an increased risk for nondisjunction when the number of genome-wide recombination events is less than some threshold. Further studies are required to confirm these results as they are based on small numbers. Such studies would help determine the importance of genetic and environmental factors that regulate recombination and to determine their impact on nondisjunction.

#### *Maternal health and environmental factors*

Risk factors for nondisjunction can be categorized into those independent of maternal age and those associated with maternal age. Altered patterns of recombination are considered maternal age-independent, as recombination occurs during the fetal stage of the female. Previously, Lamb et al. (1996) suggested a two-step model: the first step involves the establishment of a “susceptible” exchange configuration in the fetal oocyte; the second event involves an age-dependent abnormal processing of that susceptible bivalent at metaphase I. The data reviewed

above suggest that, at least for MI errors, a medially placed exchange protects the bivalent from age-related effects.

One way to identify maternal age-related risk factors associated with nondisjunction is to examine maternal health factors and environmental exposures in mothers who had a maternally-derived error. If a factor is identified as increasing the risk for nondisjunction, it may be possible to characterize that risk in the context of aging. For example, as a woman ages, the number of follicles maturing to the preovulatory stage at each menstrual cycle decreases; only one of those follicles progresses to ovulation. Thus, a decrease in the number of maturing follicles is postulated to decrease the probability that one of them will be at the precise stage necessary for optimal response to follicle stimulation hormone (FSH), the trigger for ovulation. In 1989, Warburton put forth the "limited oocyte pool" hypothesis suggesting that under these circumstances, the follicle "selected" for ovulation may be one whose oocyte is under- or over-ripe and thus more susceptible to undergo nondisjunction. Freeman et al. (2000) tested this hypothesis directly by determining the frequency of a previous ovariectomy in mothers of infants with and without DS in a population-based study. Comparing all cases of maternal origin with controls, they found a significantly greater number of case mothers with a reduced ovarian complement (OR: 9.61; 95% CI: 1.18–46.3). These data are consistent with the "limited egg pool" hypothesis. Of course, the attributable risk due to ovariectomies is small, especially compared with the overwhelming risk factor of maternal age. However, these data show the potential of using the epidemiological data to begin to narrow the putative factors related to nondisjunction that are influenced by maternal age.

Other indirect evidence to support the limited oocyte pool hypothesis has been reported specifically related to trisomy 21. For example, van Montfrans et al. (2001) reported that mothers who had experienced a trisomy 21 pregnancy had a significantly lower birth weight for gestational age than controls. Also consistent with this hypothesis, van Montfrans et al. (1999) found higher levels of FSH among women with an infant with DS compared with controls. To date, increased levels of FSH have not been confirmed for trisomies as a group or specifically for trisomy 21 (see Warburton, 2005), thus the example only shows the potential of this approach.

Cytogenetic and epidemiological studies have identified a wealth of candidates for environmental risk factors. Smoking at the time of pregnancy is an excellent example of the difficulties and limitations of such studies. Previously, a number of studies reported a nonsignificant negative association between maternal smoking around the time of conception and the risk for DS (e.g., Kline et al., 1983, 1993; Hook and Cross, 1985, 1988; Shiono et al., 1986; Chen et al., 1999). One explanation for the negative association was that trisomic conceptuses were selectively lost prenatally among women who smoke (Kline et al., 1993; Hook and Cross, 1985). However, other studies concluded that there is no association between DS and periconceptional smoking (e.g., Cuckle et al., 1990; Kallen, 1997; Torfs and Christianson, 2000). Yang et al. (1999) analyzed periconceptional smoking among women less than 35 years of age with maternal MI and MII errors separately and found an increased frequency of smoking among women with MII errors only. The

odds ratio for this group of women increased significantly if the interaction term of periconceptional smoking and oral contraceptive use was modeled. However, similar to other studies, this analysis was limited by sample size and must be confirmed before considering possible mechanisms.

Other factors such as alcohol (e.g., Kaufman, 1983), maternal irradiation (e.g., Uchida, 1979; Strigini et al., 1990; Padmanabhan et al., 2004), fertility drugs (e.g., Boue and Boue, 1973), oral contraceptives (e.g., Harlap et al., 1979; Yang et al., 1999), spermicides (e.g., Rothman, 1983; Strobino et al., 1986), parity (reviewed by Chan, 2003) and low social economic status (Torfs and Christianson, 2003; Christianson et al., 2004) have been implicated, but not confirmed. It seems almost certain that such environmental risk factors exist. Studies from model organisms make it clear that a wide variety of genetic and environmental disturbances can affect aneuploidy levels. Large population-based studies that separate individuals with DS by type of error are in progress and will aid in the identification of risk factors that have remained elusive.

#### *Genetic factors from studies of maternal nondisjunction*

Experimental organisms have been used to identify genes that are important in the proper segregation of chromosomes. Thus, variability in genes involved in the meiotic process (e.g., homolog pairing, assembly of the synaptonemal complex, chiasmata formation, sister chromosome cohesion, meiotic spindle formation, etc.) are all candidates for predisposing chromosome nondisjunction. To date, a large study to investigate the variation in such genes for their role for nondisjunction of human chromosome 21 has not been conducted.

One candidate gene, not directly related to the meiotic process, has been examined by many groups. In 1999, James et al. (1999) provided preliminary evidence from a small case-control study in the North American that the 677C→T polymorphism in the methylenetetrahydrofolate reductase (MTHFR) gene increased the risk of having a child with DS (OR = 2.6). This polymorphism is associated with an elevation in plasma homocysteine and/or low folate status. The authors hypothesized that low folate status, whether due to dietary or genetic factors, could induce centromeric DNA hypomethylation and alterations in chromatin structure. Such alterations could adversely affect DNA-protein interactions required for centromeric cohesion and meiotic segregation. This initial report stimulated several follow-up studies of the *MTHFR* 677C→T polymorphism, as well as several other allelic variants in the folate pathway, as possible genetic risk factors for having a child with DS. James (2004a, b) provides an excellent review of these studies and shows that results are inconsistent, especially those that have evaluated genotype alone without biomarkers of metabolic phenotype. Those who have examined blood homocysteine levels, a broad-spectrum indicator of nutritional and/or genetic impairment in folate/B12 metabolism, have documented a significantly higher level among the mothers of children with DS compared with control mothers from the same country. James (2004b) suggests that one possible explanation for the inconsistent results among the numerous studies may reflect the complex interaction between effects of genetic variants and nutritional intake. She further suggests that future

studies should consider the maternal metabolic phenotype as a more sensitive indicator of risk of having a child with DS than maternal genotype alone.

Another approach to determine if genes may be involved in human nondisjunction is to examine the association of consanguinity and trisomy 21. If such an association were found, this would provide evidence for a genetic effect for nondisjunction. Alfi et al. (1980) provided one of the earlier reports suggesting an association between increased consanguinity among parents of individuals with DS in a study population in Kuwait. They postulated the existence of a gene that increases the risk for mitotic nondisjunction. Alternatively, they suggested that increased rates of consanguinity among parents would be correlated with those in grandparents and therefore, an autosomal recessive gene may be postulated to be involved in meiotic nondisjunction in the homozygous parents. Since that time, reports have found no evidence for an association between consanguinity and human nondisjunction (e.g., Devoto et al., 1985; Hamamy et al., 1990; Roberts et al., 1991; Basaran et al., 1992; Zlotogora, 1997; Sayee and Thomas, 1998; Rittler et al., 2001).

Lastly, differences in the prevalence of DS among different racial groups may provide indirect evidence for genetic factors involved in human nondisjunction. However, such studies are difficult to conduct and to interpret. Differences (or similarities) may reflect the maternal age distribution of the population, completeness of ascertainment among infants and/or prenatal diagnoses, accuracy of diagnosis, cultural preference and/or access to selective prenatal termination of pregnancies with trisomic fetuses, and as yet unidentified environmental factors. Carothers et al. (1999) reviewed all published reports that included information on maternal age and selective termination. Although they found variation, they concluded that "real" variation between population groups published to date is minimal. Most importantly, they suggest that reliable data from many populations are still lacking.

### Paternal nondisjunction

As described above, only about 6–10% of all trisomy 21 cases is due to errors in spermatogenesis. There are preliminary data to suggest that the percent of paternal cases observed among prenatal diagnoses (11%) may be higher than that estimated among live born infants (7%) (Muller et al., 2000). However, no evidence is available to support a differential survival rate of paternal versus maternal derived trisomy 21 fetuses (Zaragoza et al., 1994; Muller et al., 2000) and currently there is no evidence for imprinted genes on chromosome 21 (e.g., Rogan et al., 1999). Thus, a larger series of prenatal diagnoses will be needed to confirm this observation.

Because of the limited number of families with paternal errors, studies to identify risk factors are few. Petersen et al. (1993) were the first to distinguish the type of meiotic errors and investigate risk factors among 36 paternal cases. Combined with the data from Savage et al. (1998) who studied 67 paternal cases, several patterns evolved. First, the proportion of MI to MII errors were close to 1:1, unlike maternal cases where the ratio is closer to 3:1 (e.g., Table 1). Thus, the context in which

the chromosomes segregate presents different risk factors for nondisjunction.

Paternal age has been considered as a risk factor and investigated using several approaches. For example, investigators have recently examined paternal age in large series of infants with DS (not grouped by type of error) ascertained through surveillance systems (e.g., Stoll et al., 1998; Kazaura and Lie, 2002; Fisch et al., 2003). Conflicting results have been obtained and depend significantly on the statistical modeling approach. Another approach is to examine paternal age among only infants with DS due to MI- or MII-paternal errors. Such studies have not found a paternal age effect (Petersen et al., 1993; Savage et al., 1998). Lastly, studies have examined rates of sperm with disomy 21 by age of the donor. These data are reviewed in Buwe et al. (2005) and indicate that, although studies are not conclusive, no strong evidence is present for a paternal age effect.

### Paternal recombination

Only one study has examined recombination patterns among paternal cases, but sample sizes were limited (Savage et al., 1998). Among 22 MI cases, there was evidence for reduced recombination along the non-disjoined chromosomes 21. Thus, bivalents with no exchanges are at an increased risk for nondisjunction in sperm, similar to nondisjunction of the X/Y bivalent (Hassold et al., 1991). No difference in recombination was detected among 27 paternal MII cases as compared with controls.

Interestingly, the male:female sex ratio of infants with DS is significantly increased compared with those without DS and this increase is found to be primarily among those with paternal nondisjunction errors. This was noted first using cytogenetic polymorphisms (Nielsen et al., 1981; Mikkelsen et al., 1990; Huether et al., 1996) and later confirmed with DNA marker analysis (Petersen et al., 1993; Savage et al., 1998). Savage et al. (1998) provided preliminary evidence that this increased sex ratio was restricted to MII errors (2.5) compared with MI errors (1.0). Surprisingly, classification of MII cases based on the position of the exchange event suggested that the increased sex ratio among the offspring with DS was restricted to non-telomeric exchange cases. Again, these data are preliminary, but if confirmed, may shed light on the paternal nondisjunction process.

### Paternal health and environmental factors

To date, population-based case/control studies have had limited success in studying paternal health and environmental factors among fathers who have had an infant with trisomy 21 due to a paternal nondisjunction error. This, of course, is due to the relatively low rate of errors during spermatogenesis compared with oogenesis present in a population-based series and to the reduced rate of participation among men with and without an infant with DS (unpublished data).

### Genetic factors from studies of paternal nondisjunction

One interesting approach to identify a possible genetic risk for meiotic nondisjunction of chromosome 21 is to examine the rates of disomy among sperm of fathers who have had an infant with trisomy 21 due to a paternal error. Two such studies have

been conducted. In the study of Hixon et al. (1998), ten fathers with paternal chromosome 21 errors were evaluated using fluorescence in situ hybridization (FISH) to screen for aneuploidy in sperm. The overall frequency of disomy 21 in sperm of those ten fathers was not different from the frequency in the general male population. None of the ten fathers had significantly elevated levels of disomic sperm for any chromosome. In contrast, Blanco et al. (1998) studied two men with trisomy 21 infants due to paternal errors and found significantly increased rates of disomy 21 and, in one male, increased rates of diploidy compared with controls. Further evaluation of these two men identified increased rates of disomy for chromosomes 13 and 22 (Soares et al., 2001). These data suggest that a subset of men may be predisposed to chromosome nondisjunction.

## Summary

Significant progress has been made in understanding nondisjunction of chromosome 21, the most common cause of DS. Over the past 10 years, the genetic tools available have increased the ability to accurately separate cases into discrete groups based on the parent of origin and type of nondisjunction error. Clearly, maternal age and altered recombination remain the only well-established risk factors for nondisjunction of chromosome 21. Nevertheless, additional risk factors for this multifactorial trait will be identified and progress toward understanding the effect of maternal age on the meiotic process will be made given the advances in technology, publicly available genomic resources, and interdisciplinary approaches to these important studies.

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