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The Frequency of Aneuploidy in Cultured Lymphocytes Is Correlated with Age and Gender but Not with Reproductive History

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Summary

The clinical significance of low numbers of aneuploid cells in routine cytogenetic studies of cultured lymphocytes is not always clear. We compared the frequencies of chromosome loss and gain among five groups of subjects whose karyotypes were otherwise normal; these groups were (1) subjects studied because of multiple miscarriages, (2) parents of live borns with autosomal trisomy, (3) subjects studied because they had a relative with Down syndrome, (4) an age-matched control group of phenotypically normal adults studied for other reasons (e.g., parent of a dysmorphic child or member of a translocation family), and (5) other mostly younger and phenotypically abnormal subjects who could not be assigned to the first four groups (e.g., individuals with multiple congenital anomalies or mental retardation). No significant age, sex, or group effects were observed for autosomal loss (hypodiploidy) or gain (hyperdiploidy). Autosomal loss was inversely correlated with relative chromosome length, but autosomal gain was not. Sex-chromosome gain was significantly more frequent in females than in males, but sex-chromosome loss was not significantly different between the sexes. Significant age effects were observed for both gain and loss of sex chromosomes. When age and sex were accounted for, the frequencies of sex-chromosome loss and gain were not significantly different among the five clinical groups. In general, low numbers of aneuploid cells are not clinically important when observed in blood chromosome preparations of subjects studied because of multiple miscarriages or a family history of autosomal trisomy.

Introduction

Karyotype studies are done on a large number of phenotypically normal adults. These are often couples who have experienced more than one miscarriage or who are relatives of subjects with autosomal trisomy. The expectation is that some will have a balanced chromosome rearrangement. Some workers have considered the single hypodiploid or hyperdiploid cell observed during the course of the cytogenetic analysis of these subjects to be of no clinical significance (e.g., see Sandberg et al. 1967; Wenger et al. 1984; Horsman et al. 1987; Castle and Bernstein 1988; Hassold et al. 1988). But others have described a higher frequency of hyperdiploid cells or "sex chromosome mosaicism" in parents of aneuploids and in individuals with multiple miscarriages (e.g., see Stallard et al. 1981; Staessen et al. 1983; Ford 1984; Hecht et al. 1984; Holzgreve et al. 1984; Juberg et al. 1985; Sachs et al. 1985). Larger-scale investigations with age-matched controls were recommended to assess the significance of these aneuploid cells (Michels et al. 1982). Here we report our findings from a retrospective study to examine the pattern of aneuploidy against a normal karyotypic background, particularly in adults within their reproductive years.

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Material and Methods

All peripheral blood karyotypes processed over the 5-year period 1979–83 at Henry Ford Hospital and the University of Michigan were included in a retrospective study. Each karyotype record typically included data on analysis of 15–30 G-banded mitotic spreads of peripheral leukocytes cultured using standard techniques. Each karyotype record was placed in one of the five following groups: (1) multiple miscarriages, including 698 individuals who had had—or whose spouses had had—two or more spontaneous abortions, (2) parents of trisomics, including 62 parents of offspring with trisomy 21, nine parents of offspring with trisomy 13, and seven parents of offspring with trisomy 18, (3) other relatives of Down syndrome subjects whose exact karyotype was unknown to the relatives, including 161 chromosomally normal first- and second-degree relatives of unkaryotyped Down syndrome subjects, (4) control adults, constituting an age-matched comparison group and including 332 phenotypically and karyotypically normal adults (e.g., members of translocation families and parents of a karyotypically normal dysmorphic child), and (5) all others, including 1,139 mostly younger individuals who could not be assigned to one of the preceding groups (e.g., chromosomally normal subjects with multiple congenital anomalies or with mental retardation). None of the multiple miscarriage subjects had a trisomic live-born offspring. The karyotypes of their miscarriages were unknown. About 5% of the couples in the parents of trisomics group had had two miscarriages, but these subjects were all referred for chromosome analysis because of their trisomic offspring.

Subjects with abnormal karyotypes and those interpreted as having chromosomal mosaicism were excluded from the study. The mosaics included an infant with ambiguous genitalia and X/XY mosaicism; two Klinefelter patients with XY/XXY mosaicism; six Down syndrome patients with trisomy 21 mosaicism; one each with malformations and mosaicism for r(5), +8, +18, r(21), +22, or + small ring chromosome; one man with 46,XY (three cells)/47,XYY (14 cells) whose wife had had multiple miscarriages; two blood specimens to confirm prenatally diagnosed mosaicism: 46,XX/47,XXX and 45,XY,t(13;13) (p11;p11)/46,XY; and 25 females with features consistent with Turner syndrome who had X numerical mosaicism, X/XY mosaicism, X isochromosomes, rings, deletions, translocations, and a familial small supernumerary ring chromosome.

The karyotype data sheets were reviewed to identify hypodiploid or hyperdiploid cells. Next we confirmed, microscopically or from photographs, which chromosomes were lost or gained. A cell was defined as hyperdiploid if it had more than 46 structurally normal chromosomes and if the extra chromosome(s) was recognizable and structurally normal. Cells were excluded which were found to be "hyperdiploid" because of gain of a chromosome which differed greatly in the degree of condensation compared with that in the remaining chromosomes in the metaphase spread, since such a chromosome usually represents a "floater" from a broken metaphase cell (i.e., a technical artifact). Extra X chromosomes with "premature centromere division" were not excluded (Fitzgerald et al. 1975). If a subject had a cell with sex-chromosome aneuploidy, our routine laboratory procedures dictated counting 35–50 additional cells whenever possible. For statistical analysis reasons these additional cells were not included in the study.

Statistical tests were performed on individuals rather than on cells. Since a varying number of cells were studied per individual (range 3–99, mean 20, median 16 cells/person), Mantel-Haenszel χ^2 tests were used to compare the presence or absence of hypo- and hyperdiploid cells among groups, separately for each sex after subjects were stratified by number of cells scored. Strata used were <10, 11–15, 16–20, 21–25, and ≥ 26 cells scored. Mantel-Haenszel tests were also used to compare males and females for sex-chromosome hypo- and hyperdiploidy, again with adjustment for the number of cells scored. A weighted regression analysis was also used to test for a group effect on hyper- or hypodiploidy, after adjustment for age, age squared, and sex. For all regression analyses the proportion of cells with the finding of interest was computed for each person, and this proportion was used as the dependent measure being studied. An arcsin transformation (Neter and Wasserman 1974) of the proportions was used. By using a proportion for each subject and using a weighted regression analysis, it was possible to adjust for the differing number of cells studied per person. Age squared was included because plots suggested some findings were not linearly related to age. The five clinical groups were represented in the regression model by four dummy variables. The dummy variables were formulated such that the group "all others" would be represented by a negative response to membership in any of the other four groups. Where no group effect was detected, a stepwise regression analysis was run with age, age squared, and sex as the independent variables

to assess the effects of these variables. Because of the many analyses, a *P*-value of .01 was used as the criterion for significance of a group effect in the regression analyses and for entry in the stepwise analysis. The regression analysis statistics are summarized in the Appendix.

Results

In all, 47,361 cells were analyzed from 2,408 subjects (tables 1 and 2). At least one cell with autosomal hypodiploidy was found in 39.7% of the karyotype studies, and 2.8% of all female cells and 3.0% of all male cells had loss of an autosome (not a significant sex difference; $P > .53$). No significant differences among clinical groups were found for males ($P > .58$) or females ($P > .70$) in the frequency of autosomal hypodiploidy. No significant group ($P > .25$), age, or sex effects were observed when regression analysis was used (see Appendix). The distribution of this random loss was inversely correlated ($R = -.78$; fig. 1) with the relative length of each chromosome (Van Dyke et al. 1986).

Autosomal hyperdiploidy was encountered in at least one cell from 2.1% of females and 2.1% of males, accounting for 0.11% of all cells (tables 1 and 2). There was low correlation between relative chromosome length and frequency of hyperdiploidy of individual chromosomes ($R = .04$; fig. 1). No significant differences were found among the groups in the distributions of autosomal hyperdiploidy in either males or females ($P > .19$ and $P > .43$, respectively). No significant group ($P > .18$) and no age or sex effects were detected when regression analysis was used. Only two of the parents of trisomics had cells with an extra autosome. Subject B79–267, the 22-year-old father of an individual with trisomy 21, had one cell among 14 with 47,XY,+19. This was his first child. Subject B79–71, a 26-year-old mother of a trisomy 21, had one cell among 20 with 48,XX, +10,+11. Three of the next 70 cells scored had autosomal trisomy: +3, +10, and +19. She had three other children, all normal. In no instance was there evidence of parental mosaicism for the trisomy present in the probands. Appendix table A1 provides a grid stratified by age and number of cells scored for the parents of trisomics.

Sex-chromosome loss was observed in at least one cell from 4.2% of female and 3.0% of male subjects, accounting for 0.24% of all female and 0.17% of all male cells (not a significant sex difference; $P > .54$). Sex-chromosome effects were evaluated by testing total sex-chromosome loss (or gain) and by testing the X and Y separately. For males, no significant differences were found among groups in the distribution of X or Y loss ($P > .44$ and $P > .49$, respectively). For Y chromosome

Table 1

Number of Aneuploid Cells Observed among Males and Females in the Five Clinical Groups

Group	No. of Subjects	Mean Age (years)	No. of Cells						
				Hypodiploid			Hyperdiploid		
			Total	−Autosome	−X	−Y	+Autosome	+X	+Y
Females:									
All others	592	10.4	13,526	401	15		11	7	
Control adults	174	30.1	3,032	77	16		3	6	
Multiple miscarriage	378	29.9	7,469	195	26		13	23	
Parents of trisomics	43	29.5	783	21	3		1	1	
Relatives of Down syndrome individuals	109	27.9	1,802	55	3		1	5	
Total females	1,296		26,612	749	63		29	42	
Males:									
All others	547	9.8	11,116	341	7	13	9	0	0
Control adults	158	31.8	2,552	75	4	1	4	1	1
Mutiple miscarriage	320	31.4	5,696	158	3	6	9	1	2
Parents of trisomics	35	28.8	516	21	0	0	1	0	0
Relative of Down syndrome individuals	52	28.2	869	34	0	2	0	0	0
Total males	1,112		20,749	629	14	22	23	2	3

Table 2

Subjects in the Five Clinical Groups Who Had One or More of Each Type of Aneuploid Cell

Group	No. of Subjects	No. (%) of Hypodiploid Cells			No. (%) of Hyperdiploid Cells		
		− Autosome	− X	− Y	+ Autosome	+ X	+ Y
Females:							
All others	592	258 (43.6)	15 (2.5)		11 (1.9)	6 (1.0)	
Control adults	174	55 (31.6)	12 (6.9)		3 (1.7)	5 (2.9)	
Multiple miscarriage	378	143 (37.8)	21 (5.6)		11 (2.9)	19 (5.0)	
Parents of trisomics	43	17 (39.5)	3 (7.0)		1 (2.3)	1 (2.3)	
Relatives of Down syndrome individuals	109	41 (37.6)	3 (2.8)		1 (.9)	5 (4.6)	
Total females	1,296	514 (39.7)	54 (4.2)		27 (2.1)	36 (2.8)	
Males:							
All others	547	228 (41.7)	7 (1.3)	11 (2.0)	9 (1.6)	0	0
Control adults	158	55 (34.8)	4 (2.5)	1 (.6)	4 (2.5)	1 (.6)	1 (.6)
Multiple miscarriage	320	120 (37.5)	3 (.9)	6 (1.9)	9 (2.8)	1 (.3)	2 (.6)
Parents of trisomics	35	13 (37.1)	0	0	1 (2.9)	0	0
Relatives of Down syndrome individuals	52	25 (48.1)	0 (1.3)	2 (3.8)	0	0	0
Total males	1,112	441 (39.7)	14 (1.3)	20 (1.8)	23 (2.1)	2 (.2)	3 (.3)

loss no age or group effects were detected by regression analysis ($P > .73$). For females there was a significant difference among groups for "X chromosome loss" ($P < .001$ by χ^2, not accounting for age effects). The full regression analysis model revealed no significant difference in X chromosome loss among the clinical groups ($P > 0.02$) after accounting for the age and sex effects. The subsequent stepwise regression analysis revealed significant age squared and sex effects for X chromosome loss. The regression analysis for "sex chromosome

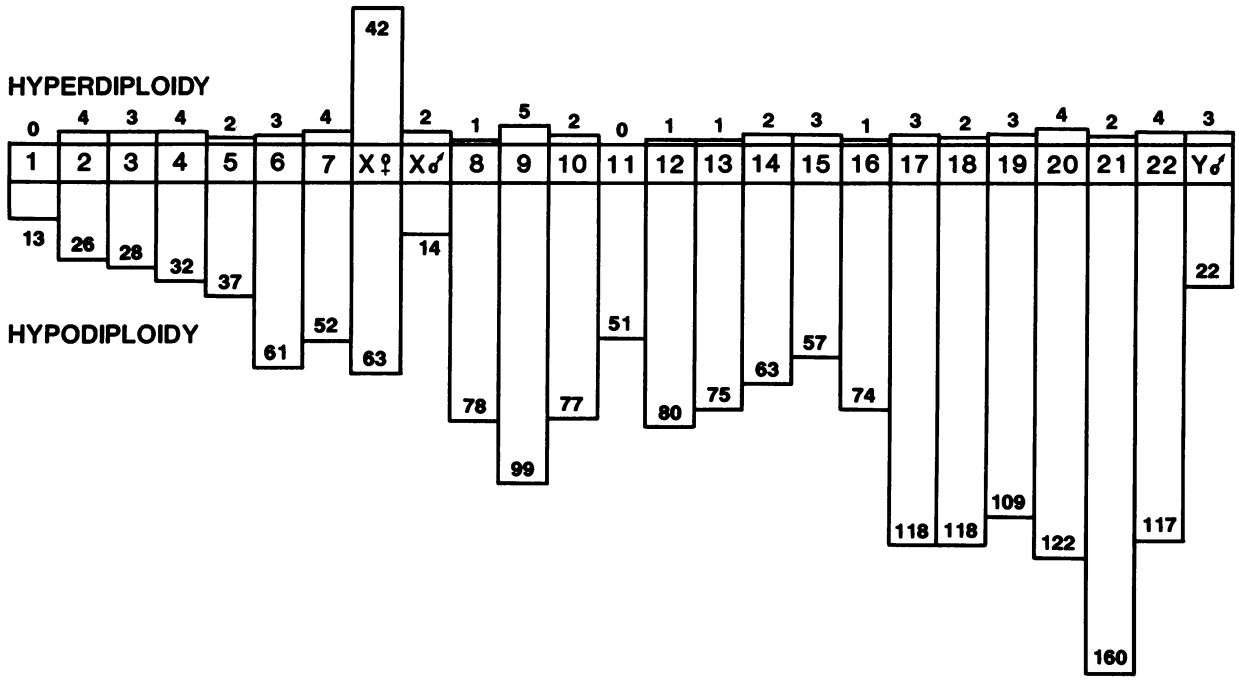

Figure 1 Distribution, by chromosome, of hypo- and hyperdiploidy in the 47,361 metaphase cells. The length of each bar and the number at the end of each bar represents the number of cells showing gain or loss of that chromosome.

Table 3

Proportion of Females with X Aneuploid Cells among the 15–30 cells Scored for Each Subject

Group	No. of Females	% Having One or More Cells with X Chromosome Loss	% Having One or More Cells with X Chromosome Gain
Under 23 years of age	538	1.7	0
23–34 years of age	577	5.2	4.0
35–44 years of age	123	9.8	8.9
Over 44 years of age	14	21.4	14.3
All females	1,252	4.4	2.9

NOTE.—The females are grouped into four age groups. The total number of females included in this table is 44 fewer than in tables 1 and 2 because we were unable to learn the exact ages of some patients. One 30-year-old woman (case B83-103) with four 45,X cells was karyotyped because she had had one miscarriage and two stillbirths. Four of the first 11 cells examined were 45,X. These four cells were found on the same microscope slide within a 1.7 × 1.4-mm rectangle. These were interpreted as representing a single in vitro colony because of their proximity to each other and because no other 45,X cells were found in a further 68 cells, all from the same cell culture, scored on two microscope slides. A similar observation was made by de la Chapelle (1982). Six females had two monosomy X cells, and 47 had a single monosomy X cell. One 30-year-old woman (case B81-519) karyotyped for multiple miscarriage had, among 30 cells scored, one cell with trisomy X and two cells with tetrasomy X. A follow-up analysis on this patient in 1983 (B83-267) revealed, among 100 lymphocyte metaphase cells, four monosomy X cells and two trisomy X cells. This was interpreted as being within normal limits. Four other women had two trisomy X cells, and 31 women had a single trisomy X cell.

loss" detected no group effect ($P > .65$), again detected an age effect (age squared) for sex-chromosome loss, but detected no sex effect. The number of females who had cells with X chromosome loss or gain is stratified by age in table 3.

Sex-chromosome gain was found in at least one metaphase cell from 2.8% of female and 0.4% of male subjects, accounting for 0.16% of all female and 0.02% of all male cells ($P < .001$). In females, the X chromosome was gained more frequently than other chromosomes of comparable size ($P < .001$), whereas in males, when the frequencies of X and Y gain were compared with gains for autosomes of comparable size, a difference could not be detected. In males, there were no significant differences among groups for X chromosome gain ($P > .21$). Differences were detected among groups for Y chromosome gain ($P < .001$), but since only three +Y cells were observed the biological significance of this finding is uncertain (table 2), and the regression analysis detected no group effects for Y chromosome gain ($P > 0.11$) and no age effects. In females, there was a highly significant difference among the groups for X chromosome hyperdiploidy ($P < .001$ by χ^2, not accounting for age effects). After age and sex were accounted for, there was no significant differences among the clinical groups in X chromosome gain ($P > .32$) or sex-chromosome hyperdiploidy ($P > .27$). The subsequent stepwise regression analysis detected significant age (age squared) and sex effects.

Discussion

Jacobs et al. (1961) reported an increased frequency of hypodiploidy with increasing age in normal individuals. This effect was greater in females, and the age and sex differences were accounted for by loss of a C-group chromosome in females and of a G-group chromosome in males (Jacobs et al. 1963, 1964; Court Brown et al. 1966). Hyperdiploidy also increased with age and in females was attributed to 47,+C cells. Most subsequent nonbanded studies confirmed an excess of 45,–C cells in females and of 45,–G cells in males, especially after age 45 years in females and after age 55 years in males (e.g., see Hamerton et al. 1965; Sandberg et al. 1967; Nielsen 1968, 1970; Jarvik et al. 1974; Mattevi and Salzano 1975; Fitzgerald and McEwan 1977). One 6-year study demonstrated an increasing frequency of hypo- and hyperdiploidy with age in elderly female twins but not in elderly male twins (Jarvik and Kato 1970; Jarvik et al. 1976). Pierre and Hoagland

(1971, 1972) reported an age-related loss of the Y from peripheral blood and bone marrow metaphase cells of healthy elderly males. The first large study of aneuploidy and age in the chromosome-banding era confirmed significant age and sex effects (Galloway and Buckton 1978). Hypodiploidy was more common in older individuals and was more evident in females, because of an excess of 45,X cells. The Y was lost more often in older males than in younger males. Hyperdiploidy, mainly 47,XXX, increased with age in females. Dramatic within-subject variation was observed when studies were repeated. Ford and Russell (1985) confirmed that X aneuploidy increases with age, and Abruzzo et al. (1985) found that in women this could be accounted for entirely by aneuploidy of the late-replicating X.

One group found an increased frequency of hyperdiploid cells in parents of aneuploid children (Stallard et al. 1981). Ford (1984) described a higher ratio of hyperdiploid cells to hypodiploid cells in mothers of Down syndrome patients than in comparison groups, even though the total frequency of aneuploid cells was not increased. Others reported increased sex-chromosome hyperdiploidy or total hyperdiploidy in couples (mostly the females) with multiple miscarriages (Stassen et al. 1983; Hecht et al. 1984; Holzgreve et al. 1984; Juberg et al. 1985; Sachs et al. 1985). Some reports dealing with the association between balanced chromosome rearrangements and multiple miscarriages also described "sex chromosome mosaicism" in such couples (Michels et al. 1982; Osztovics et al. 1982; Diedrich et al. 1983; Pantzar et al. 1984; Campana et al. 1986). One line of reasoning has been that mitotic hyperdiploidy is correlated with meiotic nondisjunction and that the appearance of a hyperdiploid cell might indicate a genetic predisposition to nondisjunction, putting such individuals at higher risk for aneuploid live borns and spontaneous abortions. If so, then individuals with hyperdiploid cells discovered in a routine chromosome analysis should be offered prenatal diagnosis in future pregnancies. However, only a few studies have employed age- or sex-matched comparison groups or have had sample sizes sufficient to attain statistical significance in comparing the frequency of aneuploid cells among groups of subjects who were in their reproductive years. In the present study, we did not find among the four groups of adults any differences in the frequency of autosomal or sex-chromosome loss or gain.

We found no significant age or sex effects on the frequency of autosomal loss or gain. Our observations confirm that the frequency of autosomal aneuploidy does not increase with advancing age (Ford and Russell 1985) and that loss (but not gain) of autosomes is inversely correlated with chromosome length (e.g., see Neurath et al. 1970; Nicholls et al. 1978; Smith and Elliott 1980; Stallard et al. 1981; Brown et al. 1983; Wenger et al. 1984).

Of the 20 males with 45,X,–Y cells, 18 had one such cell and two males had two cells with 45,X,–Y. We analyzed additional cells from these two chromosome preparations, and in one, from a 15-year-old referred to rule out Klinefelter syndrome, we identified a low frequency of abnormal cells: 45,X/46,XY/46,X,r(Y). No statistically significant increase of 45,X,–Y cells in older men was evident in the present study, perhaps because this age effect occurs beyond the reproductive years.

We confirmed that X aneuploidy in peripheral blood chromosome preparations of females increases with advancing age. Sex-chromosome losses and gains were significantly more frequent in adult (age 18 years or older) females than in adult males, but we detected no differences between the younger male and female groups. Thus sex and age effects were evident and were interacting ($P < .001$, by regression analysis interaction term). This age effect in females is evident well within the reproductive years, so it needs to be considered when one interprets the clinical significance of X aneuploid cells in routine karyotype studies. We observed cells with X chromosome loss or gain in 5.2% and 4.0% of females age 23–34 years, but fewer than 2% of the females under age <23 years had a 45,X cell, and none under age 23 years had a 47,XXX or other X hyperdiploid cell (table 3). While we continue to interpret clinical cytogenetic results on a case-by-case basis, it is evident on empirical grounds, for example, that a single 47,XXX cell in a 10-year-old female is more suspect than the same finding in a 30-year-old female.

We conclude that a low frequency of aneuploid cells in an adult under age 35 years does not signify an increased risk of meiotic nondisjunction and is not an indication for prenatal diagnosis. Most low-level sex-chromosome "mosaicism" probably reflects age-related increases in mitotic nondisjunction and perhaps accumulation of viable aneuploid clones. Our findings are consistent with those of Meyers et al. (1986) and Horsman et al. (1987), who detected no difference between the frequency of X aneuploidy in cultured lymphocytes from women with multiple miscarriages and that in an age-matched comparison group. Furthermore, X aneuploidy was not found in cultured fibroblasts from women who had X aneuploid cells in their lymphocyte chromosome preparations. Hassold et al. (1988) ad-

dressed the issue directly and found no correlation—even in two women (ages 35–39 years at the time of their miscarriages) who had 8% aneuploid cells in their peripheral blood chromosomes—between the type of fetal trisomy and the type of aneuploidy observed in the parents' peripheral blood chromosomes.

Thus age appears to be the major variable that influences (1) the frequency of mitotic sex-chromosome aneuploidy in cultured lymphocyte chromosome preparations, (2) the frequency of meiotic aneuploidy among both live borns and miscarriages, and even (3) the total rate of spontaneous abortion (Alberman 1987). Once age is accounted for, little or no relationship remains between mitotic aneuploidy and risk of meiotic nondisjunction. Nevertheless, a very few individuals appear to be at a higher risk of having aneuploid conceptions, regardless of their age. Parental +21 mosaicism has been implicated in up to 3% of trisomy 21 live births (Harris et al. 1982). One 28-year-old woman who had three of four conceptions with trisomy 21 appeared to have low-level 47,XX,+21 and 47,XX,+18 mosaicism herself and had a high proportion of cultured lymphocytes and fibroblasts with premature centromere division involving the X, 18, and 21 chromosomes (Fitzgerald et al. 1986). Hook and Cross (1983) published "preliminary and tentative" findings that women under age 20 years who experience multiple miscarriages may have an increased risk of a Down syndrome live born in subsequent pregnancies (the sample size of interest was very small: two Down syndrome live births to 483 women under age 20 years who had had two or more abortions). It is also possible that hormonal imbalance or aberrations in ovarian physiology can increase the likelihood of meiotic nondisjunction (Bond and Chandley 1983, page 72–73). Experimental evidence examining the rate of meiotic aneuploidy in unilaterally ovariectomized mice is consistent with that view (Brook et al. 1984). Thus, although chronological age is easier to measure, it may be that biological age (e.g., years until menopause) is a more important determinant of the risk of meiotic nondisjunction and offers a fertile area of investigation.

Appendix

Table A1

Frequency of Parents of Trisomics by Age When Karyotyped, Number of Cells Counted, and Distribution of +Autosome, +X, and −X Cells

Age (years)	No. of Subjects, by No. of Cells Counted				Total No. of Subjects	No. of Subjects with Aneuploid Cells		
	4–9	10–14	15–19	20–90		+Autosome	+X	−X
Unknown	1	0	1	4	6	0	0	0
18–24	2	4	12	1	19	1	0	0
25–29	2	4	12	3	21	1	0	1
30–34	4	4	7	4	19	0	1[a]	1[a]
35–52	3	1	7	2	13	0	0	1

[a] Both cells are from among 25 cells scored from subject 03039, the 32-year-old mother of a trisomy 21, who had one other, nontrisomic child. A further 40 cells scored revealed no other X aneuploid cells.

Table A2

Regression Analysis Statistics for the Full-Regression Model and for the Final Stepwise Regression, for Each Parmeter Tested

Model and Variable[a]	Parameter Estimate	Standard Error	*P*-Value of Test of HO: Parameter = 0
Full-regression model for total hypodiploidy:			
Intercept	.23595	.01271	.0001
Age	.00017	.00141	.9021
Age squared	−.00001	.00003	.8656
Sex	.01191	.01188	.3162
MM group dummy	−.00471	.01833	.6917[b]
PT group dummy	−.00425	.03794	
RD group dummy	.02147	.02777	
CA group dummy	−.02247	.02324	
Final stepwise-regression model for total hypodiploidy:			
(No variables met the .01 significance level for entry into the model.)			
Full-regression model for total hyperdiploidy:			
Intercept	.01111	.00426	.0092
Age	.00032	.00047	.4943
Age squared	.00001	.00001	.3492
Sex	−.01334	.00398	.0008
MM group dummy	.01647	.00614	.0622[b]
PT group dummy	.00772	.01272	
RD group dummy	−.00215	.00931	
CA group dummy	.00605	.00779	
Final stepwise-regression model for total hyperdiploidy:			
Intercept	.00864		
Age	.00101	.00014	.0001
Sex	−.01322	.00397	.0009
Full-regression model for autosomal hypodiploidy:			
Intercept	.22700	.01249	.0001
Age	.00046	.00138	.7391
Age squared	−.00002	.00003	.4871
Sex	.01816	.01167	.1199
MM group dummy	−.01746	.01801	.2472[b]
PT group dummy	−.01105	.03726	
RD group dummy	.02044	.02727	
CA group dummy	−.03272	.02282	
Final stepwise-regression model for autosomal hypodiploidy:			
(No variables met the .01 significance level for entry into the model.)			
Full-regression model for autosomal hyperdiploidy:			
Intercept	.00524	.00317	.0987
Age	.00048	.00035	.1707
Age squared	−.00001	.00001	.1497
Sex	.00142	.00297	.6332
MM group dummy	.00778	.00458	.1788[b]
PT group dummy	.01056	.00947	
RD group dummy	−.00616	.00693	
CA group dummy	.00394	.00580	
Final stepwise-regression model for autosomal hyperdiploidy:			
(No variables met the .01 significance level for entry into the model.)			

(*continued*)

Table A2 (continued)

Model and Variable[a]	Parameter Estimate	Standard Error	*P*-Value of Test of HO: Parameter = 0
Full-regression model for sex-chromosome hypodiploidy:			
Intercept	.01514	.00429	.0004
Age	−.00033	.00047	.4812
Age squared	.00003	.00001	.0224
Sex	−.00709	.00401	.0770
MM group dummy	.00596	.00618	.6511[b]
PT group dummy	.00639	.01279	
RD group dummy	−.00389	.00936	
CA group dummy	.01296	.00784	
Final stepwise-regression model for sex-chromosome hypodiploidy:			
Intercept	.01076		
Age squared	.00002	.00000	.0001
Full-regression model for sex-chromosome hyperdiploidy:			
Intercept	.00595	.00295	.0440
Age	−.00010	.00033	.7493
Age squared	.00002	.00001	.0051
Sex	−.01524	.00276	.0001
MM group dummy	.00839	.00426	.2685[b]
PT group dummy	−.00370	.00881	
RD group dummy	.00315	.00645	
CA group dummy	.00128	.00540	
Final stepwise-regression model for sex-chromosome hyperdiploidy:			
Intercept	.00601		
Age squared	.00002	.00000	.0001
Sex	−.01542	.00275	.0001
Full-regression model for −X:*			
Intercept	.01152	.00288	.0001
Age	−.00040	.00031	.2058
Age squared	.00002	.00001	.0058
Sex	−.01150	.00270	.0001
MM group dummy	−.00525	.00416	.0284[b]
PT group dummy	.00830	.00861	
RD group dummy	−.00407	.00630	
CA group dummy	.01498	.00527	
Final stepwise-regression model for −X			
Intercept	.00955		
Age squared	.00002	.00000	.0001
Sex	−.01128	.00268	.0001
Full-regression model for +X:			
Intercept	.00468	.00206	.0232
Age	−.00012	.00023	.6084
Age squared	.00002	.00001	.0016
Sex	−.01177	.00193	.0001
MM group dummy	.00467	.00297	.3196[b]
PT group dummy	−.00300	.00615	
RD group dummy	.00184	.00450	
CA group dummy	−.00102	.00376	
Final stepwise-regression for +X:			
Intercept	.00431		
Age squared	.00002	.00000	.0001
Sex	−.01182	.00191	.0001

(*continued*)

Table A2 (continued)

Model and Variable[a]	Parameter Estimate	Standard Error	*P*-Value of Test of HO: Parameter = 0
Full-regression model for −Y:			
Intercept	.00428	.00189	.0239
Age	.00012	.00023	.5991
Age squared	−.00000	.00001	.9156
MM group dummy	−.00214	.00302	.7300[b]
PT group dummy	−.00735	.00625	
RD group dummy	−.00039	.00457	
CA group dummy	−.00521	.00383	
Final stepwise-regression model for −Y: (No variables met the .01 significance level for entry into the model.)			
Full-regression model for +Y:			
Intercept	−.00001	.00070	.9884
Age	.00001	.00009	.9428
Age squared	−.00000	.00000	.9128
MM group dummy	.00246	.00111	.1088[b]
PT group dummy	.00003	.00231	
RD group dummy	.00002	.00169	
CA group dummy	.00291	.00141	
Final stepwise-regression model for +Y: (No variables met the .01 significance level for entry into the model.)			

[a] Group designations are as follows: MM = multiple miscarriages; PT = parents of trisomics; RD = relatives of Down syndrome individuals; and CA = control adults.
[b] For test of an overall group effect.

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