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Sex Ratio in Normal and Disomic Sperm: Evidence That the Extra Chromosome 21 Preferentially Segregates with the Y Chromosome

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Summary

In humans, deviations from a 1:1 male:female ratio have been identified in both chromosomally normal and trisomic live births: among normal newborns there is a slight excess of males, among trisomy 18 live borns a large excess of females, and among trisomy 21 live borns an excess of males. These differences could arise from differential production of or fertilization by Y- or X-bearing sperm or from selection against male or female conceptions. To examine the proportion of Y- and X-bearing sperm in normal sperm and in sperm disomic for chromosomes 18 or 21, we used three-color FISH (to the X and Y and either chromosome 18 or chromosome 21) to analyze >300,000 sperm from 24 men. In apparently normal sperm, the sex ratio was nearly 1:1 (148,074 Y-bearing to 148,657 X-bearing sperm), and the value was not affected by the age of the donor. Certain of the donors, however, had significant excesses of Y- or X-bearing sperm. In disomy 18 sperm, there were virtually identical numbers of Y- and X-bearing sperm; thus, the excess of females in trisomy 18 presumably is due to selection against male trisomic conceptions. In contrast, we observed 69 Y-bearing and 44 X-bearing sperm disomic for chromosome 21. This is consistent with previous molecular studies, which have identified an excess of males among paternally derived cases of trisomy 21, and suggests that some of the excess of males among Down syndrome individuals is attributable to a nondisjunctional mechanism in which the extra chromosome 21 preferentially segregates with the Y chromosome.

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Introduction

Studies of the proportion of males to females at birth (i.e., the secondary sex ratio) in humans indicate a small but significant excess of males, with the most commonly cited male:female ratio in Caucasians being 1.06 (Visaria 1967; James 1987). Significant deviations from a 1:1 secondary sex ratio also have been reported for a number of chromosome abnormalities, most notably for certain of the trisomies. For example, among newborn trisomy 21 babies there is an excess of males, and the largest studies indicate a male:female ratio of ~1.15–1.25 (Huether 1990; Huether et al. 1996). In trisomy 18 the opposite situation pertains, since affected newborns are 1.5–4 times as likely to be female as male (Huether et al. 1996).

The effects on secondary sex ratio could arise by several mechanisms: (1) deviations in the gametic sex ratio—that is, either differential production of Y- or X-bearing sperm in meiosis or differential survival of Y- or X-bearing sperm in spermiogenesis; (2) deviations in the primary sex ratio—that is, differential fertilizing ability of Y- or X-bearing sperm; or (3) selection against male or female conceptuses. The basis of the deviations in sex ratio for chromosomally normal live births and trisomies 18 and 21 is unclear. For normal live births, it frequently has been suggested that the male bias originates at the time of fertilization, possibly in response to maternal gonadotrophin levels (e.g., see James 1980, 1987). For trisomy 21 conceptuses, it has been hypothesized that the skewing arises in meiosis, since paternally derived cases of meiotic origin show a highly significant male bias whereas corresponding maternally derived cases do not (e.g., see Petersen et al. 1993). For trisomy 18 conceptuses, it seems likely that the female bias is due to differential in utero selection, since the large female excess observed in live births is not reflected in trisomy 18 spontaneous abortions (Hassold et al. 1983; Huether et al. 1996) and because molecular studies of chromosome 18 nondisjunction indicate that paternally derived cases originate from a postmeiotic error (Fisher et al. 1995).

FISH provides an approach to the analysis of the gametic sex ratio in normal sperm and in those which

have undergone nondisjunction at meiosis I or II. In the present study we used three-color FISH with probes for the X and Y chromosomes and either chromosome 18 or 21 to analyze >350,000 sperm from 24 donors to determine whether (1) the gametic sex ratio in normal sperm deviated significantly from unity, either in our study population or in any of the individual donors, and (2) the gametic sex ratio in sperm disomic for chromosomes 18 or 21 deviated significantly from unity.

Subjects and Methods

Study Population

Semen samples were obtained from 24 healthy volunteer sperm donors. Twelve were volunteers from the Case Western Reserve University or Emory University faculty and staff; 10 were sperm donors of the University Hospitals of Cleveland Sperm Bank; and 2 were patients attending the University Hospitals of Cleveland In Vitro Fertilization Clinic for reasons unrelated to male infertility; all donors were Caucasians. Detailed reproductive histories were not obtained; however, none of the donors was known to be infertile.

Sample Processing

Semen samples were processed as described elsewhere (Griffin et al. 1995). In brief, samples were washed in a buffer solution (10 mM Tris HCl, 10 mM NaCl, pH 8), smeared onto clean microscope slides, and dehydrated in an alcohol series. After being allowed to air-dry, sperm heads were swelled by successive incubations in 0.01 M DTT and 0.01 M LIS (diidosalicylic acid and lithium salt). Slides were dehydrated in alcohol and air-dried for subsequent FISH studies.

FISH

Probes for centromeric alpha-satellite sequences of chromosomes 18 and X and for satellite III sequences in distal Yq were directly labeled by nick-translation (with FluoroRed™ or FluoroGreen™; Amersham) or were purchased commercially (Vysis); in a few cases, (Sp11–Sp15) biotinylated and digoxigenin-labeled probes (Oncor) were used. For detection of chromosome 21, a combination of a Spectrum Orange–labeled chromosome 21–specific probe (Vysis) and the D21S55 digoxigenin-labeled probe (Oncor) were used. Hybridization and three-color fluorescent detection were performed as described elsewhere (Griffin et al. 1995), and the sperm were analyzed with Zeiss Axiophot or Axioplan epifluorescence microscopes.

Analysis of Aneuploidy

Two separate experiments were conducted, one in which the sex ratio in normal and disomy 18 sperm was determined and one in which the sex ratio in disomy 21 sperm was determined. For detection of X- and Y-bearing sperm in normal and disomy 18 sperm, preparations were scored by use of a triple-bandpass filter (Chroma-Tech) for simultaneous detection of red (X chromosome), green (Y chromosome), and yellow (chromosome 18) signals, with blue total DNA background. For detection of X- and Y-bearing sperm in disomy 21 sperm, the chromosome 21 probes were labeled in red, and the X and Y chromosomes in green and blue, respectively. To eliminate observer bias in scoring of disomy 21 sperm, a sperm was identified as carrying two chromosomes 21 on a single-bandpass red filter (Zeiss) before examination on the triple-bandpass filter, to determine which sex chromosome it was carrying.

Each case was analyzed by two or three independent observers. We scored for disomy but not nullisomy, since failure to detect a signal could be due to technical difficulties as well as to nondisjunction. Stringent criteria were applied before a sperm head was classified as disomic: the two signals had to be (*a*) of equal intensity, (*b*) separated from one another by at least one signal domain, (*c*) regular in appearance and not diffuse, and (*d*) clearly positioned within the sperm head. Sperm that were not scored as disomic or diploid were considered to be normal, although a small proportion could have been disomic for another chromosome.

Statistical Analysis

Initially, statistical evaluations of possible deviations from 1:1 male:female ratios were performed by use of simple goodness-of-fit tests. For disomy 18 sperm and disomy 21 sperm, results from the individual donors were pooled, and a single analysis was conducted.

For normal sperm, an analysis of sex ratio in the entire donor set was conducted, and, in a second analysis, deviations from 1:1 ratios were evaluated for each of the 24 donors, by use of the Bonferroni correction to account for the number of individuals tested (i.e., by setting the significance level at .05/24 = .002). Two additional analyses were conducted to test for homogeneity among the donors; in the first, a simple χ^2 test of homogeneity for the whole group was performed, and, in the second test, possible individual deviations from the group's average sex ratio were evaluated, by use of the Bonferroni correction. Finally, we used weighted regression to determine whether the gametic sex ratio was affected by the age of the donor.

Results

Among the normal sperm, we scored a total of 296,731 sperm, with the ratio of Y- to X-bearing sperm being almost unity (.996) (table 1). Among individual donors, five males (Sp11, Sp14, Sp17, Sp41, and Sp49) had sex ratios significantly different (at the .05 level)

Table 1

Summary of Studies of Sex Ratio in Normal Sperm

Donor	Age of Donor (years)	No. of Y-Bearing Sperm	No. of X-Bearing Sperm	Y:X Ratio	Deviation from Sex Ratio of (χ^2 [a]) 1:1	Deviation from Sex Ratio of (χ^2 [a]) .996:1
Sp1	48	10,075	10,140	.99	NS	NS
Sp11	42	4,894	5,148	.95	6.42 ($P \approx .01$)	5.43 ($P \approx .02$)
Sp12	25	5,035	5,007	1.01	NS	NS
Sp13	43	7,304	7,341	1.00	NS	NS
Sp14	43	4,756	5,007	.95	6.45 ($P \approx .01$)	5.47 ($P \approx .02$)
Sp15	35	7,493	7,501	1.00	NS	NS
Sp16	24	5,012	4,956	1.01	NS	NS
Sp17:[b]						
1	22	4,756	5,190	.92		
2		2,377	2,595	.92		
Sp17 overall		7,133	7,785	.92	28.10 ($P < .00001$)	25.91 ($P < .00001$)
Sp18	31	6,241	6,265	1.00	NS	NS
Sp19	37	6,294	6,209	1.01	NS	NS
Sp20	28	7,253	7,203	1.01	NS	NS
Sp21	31	5,034	4,943	1.02	NS	NS
Sp25	18	4,989	4,978	1.00	NS	NS
Sp26	35	5,032	4,968	1.01	NS	NS
Sp38	60	4,978	4,969	1.00	NS	NS
Sp39	36	4,988	4,974	1.00	NS	NS
Sp40	25	5,022	4,950	1.02	NS	NS
Sp41	24	6,140	5,823	1.05	8.39 ($P \approx .004$)	9.74 ($P \approx .002$)
Sp43	52	4,981	4,946	1.01	NS	NS
Sp49	24	7,356	7,679	.96	6.19 ($P \approx .01$)	5.69 ($P \approx .02$)
Sp50	27	7,526	7,347	1.02	NS	NS
Sp51	48	7,536	7,491	1.01	NS	NS
Sp52	52	5,510	5,445	1.01	NS	NS
Sp53	53	7,492	7,582	.99	NS	NS
Group						
18–29 years ($n = 10$)		55,466	55,728	1.00	NS	
30–39 years ($n = 6$)		35,082	34,860	1.01	NS	
40–49 years ($n = 5$)		34,565	35,127	.98	NS	
50–60 years ($n = 4$)		22,961	22,942	1.00	NS	
Overall		148,074	148,657	1.00	NS	

[a] χ^2 and "uncorrected" P values $<.05$ are shown (for discussion of uncorrected and corrected significance levels, see Subjects and Methods). NS = not significant.

[b] Two ejaculates were analyzed.

from 1:1. Four of these had excesses of X-bearing sperm, and one had an excess of Y-bearing sperm. The most extreme deviation was observed in Sp17, who had a significant excess of X-bearing sperm in two different ejaculates. This was the only case that deviated significantly (at the .002 level) from a 1:1 ratio, the value used to correct for the number of individuals tested (see Subjects and Methods).

As well as evaluating possible deviations from a 1:1 sex ratio, we also tested for heterogeneity among the 24 donors. An initial χ^2 test indicated significant heterogeneity ($\chi^2_{23} = 61.9$; $P < .001$). Therefore, in a subsequent analysis we asked which of the individuals had sex ratios significantly different from .996, the average sex ratio of the group. Five cases (Sp11, Sp14, Sp17, Sp41, and Sp49) were identified as being significantly different at the .05 level, but only two (Sp17 and Sp41) were significant at the "corrected," .002 level (table 1).

We found no evidence for an effect of age of the donor on sex ratio; that is, weighted linear regression indicated no obvious association ($R^2 \approx 0$; $P = .88$), and, when the study population was divided into four age groups, each group had virtually identical sex ratios, none of which was significantly different from unity (table 1).

In studies of disomic sperm, we identified a highly significant difference, in the overall rate of disomy, be-

Table 2

Summary of Studies of Incidence of Disomies 18 and 21 and of Sex Ratio in Disomic Sperm

	No. (%) of						
	Donors	Sperm Scored	Disomic	Y-Bearing Sperm	X-Bearing Sperm	Sex Ratio	χ^2
Disomy 18	24	296,713	108 (.04%)	53	55	.96	NS
Disomy 21	9	68,075	113 (.17%)	69	44	1.57	5.53 ($P \approx .02$)

tween chromosomes 18 and 21 (table 2); that is, disomy 21 was identified in 0.17% of all sperm scored, a fourfold increase over the 0.04% value observed for disomy 18 ($\chi^2_1 = 153.6$; $P < .001$). Additionally, disomy 21 sperm were significantly more likely to be Y bearing than X bearing, since >60% of all disomy 21 sperm had a Y chromosome ($\chi^2_1 = 5.53$; $P \approx .02$). No such effect was observed for disomy 18.

Discussion

The purpose of the present study was to determine whether observed variations in secondary sex ratios might arise prior to fertilization. Specifically, we wanted to determine whether the excess of males among chromosomally normal newborns was due to an increased frequency of Y-bearing sperm in ejaculates; whether the excess of males among trisomy 21 newborns reflected preferential segregation of the Y with the two chromosomes 21 in disomy 21 sperm; and whether the excess of females among trisomy 18 newborns was due to preferential segregation of the X chromosome with the two chromosomes 18 in disomy 18 sperm.

Sex Ratio in Chromosomally Normal Sperm

Three major conclusions derive from our studies of ~300,000 normal sperm from 24 donors. First, our results indicate that, in a population of normal males, the proportion of Y- to X-bearing sperm males is 1:1 or nearly so, since our overall gametic sex ratio was 0.996. Thus in most males, spermatogenesis or spermiogenesis processes Y- and X-bearing sperm similarly. These results are somewhat different than those of Spriggs et al. (1996), who identified a small but significant excess of X-bearing sperm in studies of ~50,000 sperm from five donors. Nevertheless, neither the present study nor that by Spriggs et al. (1996) observed an excess of Y-bearing sperm in the study population. Thus, it seems unlikely that variation in gametic sex ratio is an important contributor to the excess of males observed at birth, and, indeed, the gametic sex ratio that we observed is highly significantly different ($\chi^2_1 = 288.2$; $P < .001$) from the 1.06 male:female secondary sex ratio commonly cited for Caucasian populations (e.g., see Ulizzi and Zonta 1994).

Second, the gametic sex ratio approximated unity in most of our donors. The Y:X ratios ranged from 0.92 to 1.05, and in two-thirds of the donors the values were either 0.99, 1.00, or 1.01. Further, in 22 of the 24 donors the deviations from 0.996, the average gametic sex ratio of the study population, were nonsignificant at the "corrected," 002 level (see Subjects and Methods). However, in one case (Sp17) a significant excess of X-bearing sperm was observed, and this effect was seen in two different ejaculates; in a second case (Sp41) a significant excess of Y-bearing sperm was identified in a single ejaculate; and in three other cases (Sp11, Sp14, and Sp49) deviations from 0.996 were significant at the .05 level. Similarly, in a recent FISH sperm study of five males, Spriggs et al. (1996) identified one individual with a significant excess of X-bearing sperm. Thus, for a small proportion of normal males, the gametic sex ratio may differ from 1:1. In such individuals, variations in Y- or X-linked loci may affect the formation or viability of sperm—for example, through meiotic drive (e.g., see Lyttle 1993) or because of variation in genes normally expressed in the haploid state during spermiogenesis (e.g., see Chakyo and Martin-DeLeon 1992). However, before these or any other possibilities can be seriously entertained, studies of additional donors and of multiple ejaculates per donor will be necessary to confirm the existence of such individuals.

Third, we found no evidence of an effect of donor age on the gametic sex ratio. These observations are consistent with the recent report by Martin et al. (1995), who also found no association between donor age and the proportion of Y- and X-bearing sperm. Some studies of chromosomally normal newborns have reported a decrease in male babies born to older fathers (e.g., see James and Rostron 1985). Our results and those of Martin et al (1995) provide no evidence that such an effect either originates during male meiosis or results from selection during spermiogenesis.

Studies of Disomy 21 Sperm

We identified 113 disomy 21 sperm in a total of 68,075 examined, for a 0.17% frequency of disomy. This is significantly higher than the 0.04% value that we observed for disomy 18 and is higher than the

~0.1% level of disomy reported for most other autosomes in previous FISH sperm studies (e.g., see Spriggs et al. 1996). Spriggs et al. (1996) also observed a significant increase in disomy for chromosome 21, by comparison with other autosomes studied. Thus, it may be that there are mechanisms of paternal nondisjunction that are more likely to involve chromosome 21 than other autosomes.

Our results indicate that disomy 21 sperm are more likely to be Y bearing than X bearing. These findings are consistent with those of Petersen et al. (1993), who found that trisomy 21 conceptuses that resulted from paternal meiotic errors were significantly more likely to be male than female. The basis for the association between chromosome 21 nondisjunction and the presence of a Y chromosome is unclear, but two possibilities, similar to those proposed by Peterson et al. (1993), are (1) that there is aberrant exchange, at prophase I, between a chromosome 21 and the Y chromosome, leading to cosegregation at meiosis I and (2) that the two chromosomes 21 fail to pair and/or recombine and segregate against the X chromosome.

Petersen et al. (1993) suggested that, if the Y chromosome and a chromosome 21 undergo an aberrant recombinational event, failure to resolve the exchange could result in both chromosomes migrating to the same pole. If it is assumed that the X chromosome and the other chromosome 21 segregate at random, this could result in a 24,Y,+21 sperm but not in a 24,X,+21 sperm. This mechanism presupposes that neither the chromosome 21 bivalent nor the sex chromosome bivalent undergoes homologous recombination. Thus, in theory this could be tested by use of (*a*) FISH to identify disomy 21 sperm, (*b*) microdissection techniques to obtain DNA from the disomic sperm, and (*c*) single-sperm PCR assays to determine whether recombination has occurred between the two chromosomes 21 or in the pseudoautosomal region.

Hawley and Theurkauf (1993) have proposed that, in *Drosophila* females, achiasmate chromosomes find their place on the metaphase plate by migrating to the "less crowded" pole—that is, the pole containing the least amount of chromosome material. It is unclear whether a similar pairing system exists in human meiosis. However, if it does, in rare instances in which both the sex chromosomes and the chromosomes 21 are achiasmate, the larger X chromosome may orient to one pole, with the smaller chromosomes 21 and the Y chromosome segregating against it. This would result in 22,X and 24,Y,+ 21 gametes. As in the previous model, this scenario assumes absence of recombination in the pseudoautosomal region and between the chromosomes 21. Thus, PCR-based studies of disomic sperm, as outlined above, could be used to search for recombinational events between the nondisjoined chromosomes 21 and between the XY bivalent.

Regardless of the correctness of these or other meiotic models, our results and those of Petersen et al. (1993) suggest that disomy 21 sperm are more likely to be Y bearing than X bearing. This altered rate may contribute to the sex-ratio disturbance in trisomy 21 (Hassold et al. 1983; Huether et al. 1996), but it does not explain the ~1.2 sex ratio reported among trisomy 21 live borns; that is, our results pertain only to paternally derived cases of trisomy 21, which, according to molecular studies of chromosome 21 nondisjunction (e.g., see Sherman et al. 1994), constitute ≤10% of all cases of trisomy 21. If disturbances in the male gametic sex ratio were entirely responsible for the excess of males among trisomy 21 newborns, the gametic sex ratio of paternally derived cases would have to be ~4:1 to yield a secondary sex ratio of 1.2. In fact, the Y:X ratio that we observed among disomy 21 sperm was only 1.57. Thus, other factors, such as in utero selection against female trisomy 21 conceptuses, must operate to yield the 1.2 sex ratio observed among live-born trisomy 21 individuals. Since the vast majority of pregnancies with trisomy 21 conceptuses terminate spontaneously before birth, even a small selection could skew the secondary sex ratio significantly toward males.

Studies of Disomy 18 Sperm

We found no deviation from a 1:1 Y:X ratio among disomy 18 sperm. Thus, the excess of females observed among trisomy 18 live borns is presumably due to in utero selection against trisomy 18 males. This interpretation is consistent with cytogenetic studies of spontaneous abortions, in which an excess of males among trisomy 18 fetuses has been reported (Hassold et al. 1983), and with the recent study by Huether et al. (1996), which reported a significant decline in trisomy 18 males between the time of amniocentesis and birth.

Conclusions

Our results suggest that, in most males, the number of Y- and X-bearing sperm is approximately equal and indicate that the excess of males among chromosomally normal newborns is not attributable to factors that act during spermatogenesis or spermiogenesis. We observed no difference in the proportion of Y- and X- bearing sperm among those with an additional chromosome 18, but we identified a significant excess of Y-bearing sperm among those disomic for chromosome 21. It will be important to confirm these results on a larger series and, by studying the proportion of Y- and X-bearing sperm among those disomic for other autosomes, to determine whether this effect is restricted to chromosome 21 or extends to other autosomes as well.

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References

Chakyo CA, Martin-DeLeon PA (1992) The murine Rb(6.16) translocation: alterations in the proportion of alternate sperm segregants affecting fertilization in vitro and in vivo. Hum Genet 90:79–85

Fisher JM, Harvey JF, Morton NE, Jacobs PA (1995) Trisomy 18: studies of the parent and cell division of origin and the effect of aberrant recombination on nondisjunction. Am J Hum Genet 56:669–675

Griffin DK, Abruzzo MA, Millie EA, Sheean LA, Feingold E, Sherman SL, Hassold TJ (1995) Non-disjunction in human sperm: evidence for an effect of increasing paternal age. Hum Mol Genet 4:2227–2232

Hassold T, Quillen SD, Yamane JA (1983) Sex ratio in spontaneous abortions. Ann Hum Genet 47:39–47

Hawley RS, Theurkauf W (1993) Requiem for distributive segregation: achiasmate segregation in Drosophila females. Trends Genet 9:310–317

Huether C (1990) Epidemiologic aspects of Down syndrome: sex ratio, incidence, and recent impact of prenatal diagnosis. Issues Rev Teratol 5:283–316

Huether C, Martin R, Stoppelman S, D'Souza S, Bishop J, Torfs C, Lorey F, et al (1996) Sex ratios in fetal and livebirth autosomal aneuploidies. Am J Med Genet 63:382–390

James WH (1980) Gonadotrophin and the human secondary sex ratio. BMJ 281:711–712

——— (1987) The human sex ratio. I. A review of the literature. Hum Biol 59:721–752

James WH, Rostron J (1985) Parental age, parity and sex ratio in births in England and Wales, 1968–1977. J Biosoc Sci 17:47–56

Lyttle TW (1993) Cheaters sometimes prosper: distortion of mendelian segregation by meiotic drive. Trends Genet 9: 205–210

Martin RH, Spriggs E, Ko E, Rademaker AW (1995) The relationship between paternal age, sex ratios, and aneuploidy frequencies in human sperm, as assessed by multicolor FISH. Am J Hum Genet 57:1395–1399

Petersen MB, Antonarakis SE, Hassold TJ, Freeman SB, Sherman SL, Avramopoulos D, Mikkelsen M (1993) Paternal nondisjunction in trisomy 21: excess of male patients. Hum Mol Genet 10:1691–1695

Sherman S, Petersen MB, Freeman SB, Hersey J, Pettay D, Taft L, Frantzen M, et al (1994) Non-disjunction of chromosome 21 in maternal meiosis I: evidence for a maternal age-dependent mechanism involving reduced recombination. Hum Mol Genet 3:1529–1535

Spriggs EL, Rademaker AW, Martin RH (1996) Aneuploidy in human sperm: the use of multicolor FISH to test various theories of nondisjunction. Am J Hum Genet 58:356–362

Ulizzi I, Zonta LA (1994) Sex ratio and selection by early mortality in humans: fifty-year analysis in different ethnic groups. Hum Biol 66:1037–1048

Visaria P (1967) Sex ratio at birth in territories with a relatively complete registration. Eugenics Q 14:132–142