Genetics of Human Sperm

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Purpose: Chromosome abnormalities in sperm were studied by fluorescence in situ hybridization to determine the frequency and distribution of abnormalities in normal men and the effect of donor age on the frequency of abnormalities. Studies of chemotherapy and infertility patients assessed any increased risk in these populations.

Methods: Multicolor fluorescence in situ hybridization was performed on the sperm samples to assess aneuploidy frequencies for chromosomes 1, 2, 4, 9, 12, 13, 15, 16, 18, 20, 21, X, and Y as well as "sex ratios" and frequencies of diploid sperm.

Results: Most chromosomes yielded disomy estimates of approximately 0.1%, whereas the frequencies for chromosome 21 and the sex chromosomes were significantly elevated. The only chromosome to show a significant paternal age effect was YY disomy. Chemotherapy patients did not have an increased risk of aneuploid sperm 2–13 years after treatment. Infertility patients had an increased risk of disomy for chromosome 1, 13, 21, and XY.

Conclusions: Multicolor fluorescence in situ hybridization analysis allows comparison of sperm from various populations of men and has demonstrated that infertile patients have a significant increase in the frequency of aneuploid sperm.

KEY WORDS: aneuploidy; chromosome abnormality; fluorescence in situ hybridization; human sperm; infertility.

INTRODUCTION

Chromosomal anomalies are responsible for human infertility, pregnancy loss and the birth of children with mental and physical handicaps. It has only recently

become possible to study human gametes to try to elucidate some of the causes of chromosomal abnormalities.

We have studied human sperm karyotypes (by fusion of hamster oocytes with human sperm) to study normal men (e.g., Refs. 1–8) and men at increased risk of sperm chromosomal abnormalities such as translocation and inversion carriers (e.g., Refs. 9–13) and radiotherapy and chemotherapy (CT) patients (e.g., Refs. 14–17). Human sperm karyotyping using the hamster system has a number of advantages: precise banded karyotypes are obtained with complete information about all chromosomes and characterization of both numerical and structural abnormalities. However, the disadvantages are significant: the technique is extremely time-consuming and labor-intensive, few laboratories around the world have reported success, only sperm capable of fusing with hamster oocytes can be studied and sample sizes are small. Because of these limitations, simpler faster methods have been developed by exploiting molecular technology. Fluorescence in situ hybridization (FISH) analysis using chromosome-specific DNA probes has provided a rapid, accessible method for detection of chromosomal abnormalities in human sperm (18–31). Many improvements in FISH analysis have occurred during the past few years, and multicolor FISH analysis now provides an accurate and reliable method for detection of chromosome abnormalities in human sperm (32–37). In this report we will discuss examples of our use of FISH analysis for basic research in normal men as well as men at potential increased risk of chromosomal abnormalities in their sperm.

MATERIALS AND METHODS

Normal Donors

Semen samples were obtained from normal healthy men with no history of CT, radiotherapy, or chronic illness. The men were 21 to 58 years of age.

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Cancer Patients

Cancer patients with testicular tumors who had sperm samples cryopreserved before their treatment with BEP (bleomycin, etoposide, cisplatin) were recruited from the Tom Baker Cancer Clinic. Sperm samples were obtained 2 to 13 years after CT and were frozen until analysis. Cryopreserved sperm samples obtained from the same men pre- and post-BEP treatment were thawed and analyzed. We have demonstrated previously that cryopreservation of human sperm does not affect the frequency of numerical or structural chromosomal abnormalities or the "sex ratio" of sperm (5,8).

Infertility Patients

The infertile patients in this study consisted of men who were undergoing fertility investigations after having experienced at least 1 year of infertility. These men had low sperm counts (<20 million sperm/ml), reduced motility ($<40\%$ motile), and/or poor morphology ($<40\%$ normal forms). The patients were recruited from the University of Calgary Infertility Clinic. For each patient, semen samples were collected after 3 days of abstinence. Prior to any DNA analysis, a minimum of three samples was assessed to validate the original diagnosis of abnormal semen characteristics. All of the 10 men were determined to have idiopathic infertility, and information regarding childhood diseases, environmental exposures, substance abuse, radiation exposure, and prescription drug usage was determined to decrease the possibility that external factors could account for their infertility. Each patient also had a normal endocrinology profile as determined by normal levels of FSH, LH, and T.

The patients were between 28 and 46 years of age. None of the patients had any children at the time of study either naturally or through in vitro fertilization. Through routine lymphocyte karyotyping, all patients were found to have a normal 46,XY karyotype, with one patient carrying a normal variant for chromosome 15 (missing satellites).

All participants gave their informed consent prior to the study, which was reviewed by our institutional ethics committee.

RESULTS AND DISCUSSION

Normal Men

Frequency and Distribution of Chromosomal Anomalies. All chromosomes have been represented in aneu-

ploid products of conception; however, some chromosomes are observed in the aneuploid state much more frequently than others. In newborns, aneuploidies for chromosomes 13, 18, and 21 and the sex chromosomes occur most frequently, whereas in spontaneous abortions trisomy 16 is the most frequent. These different frequencies could occur because of different rates of nondisjunction for different chromosomes during meiosis or because of differential survival during embryonic development, or both. Using sperm karyotype analysis, we have demonstrated that all chromosomes are susceptible to nondisjunction during spermatogenesis but that the sex chromosome bivalent and the chromosome 21 bivalent have an increased frequency of nondisjunction compared to the other chromosomes (7). Using multicolor FISH and chromosome-specific probes, the frequency of disomy in human sperm was determined for chromosomes 1, 2, 4, 9, 12, 15, 16, 18, 20, and 21 and for the sex chromosome (38). A minimum of 10,000 sperm with signals was scored from each of five normal donors for every chromosome studied, giving a total of 418,931 sperm nuclei. The mean frequencies of disomy obtained were 0.09% for chromosome 1; 0.08% for chromosome 2; 0.11% for chromosome 4; 0.14% for chromosome 9; 0.16% for chromosome 12; 0.11% for chromosomes 15, 16, and 18; 0.12% for chromosome 20; 0.29% for chromosome 21; and 0.43% for the sex chromosomes. When the mean frequencies of disomy were compared, the sex chromosomes ($P < 0.0002$) and chromosome 21 ($P < 0.0001$) had significantly higher frequencies of disomy than of any other autosome studied. These data corroborate our results from human sperm karyotypes (7) and suggest that the sex chromosome bivalent and the chromosome 21 bivalent are more susceptible to nondisjunction during spermatogenesis. Other FISH studies on human sperm also suggest an increase in the frequency of aneuploidy for the sex chromosomes and chromosome 21 compared to the autosomes (32,33,39). Recent studies have shown that the majority of sex chromosomal aneuploidies originate as paternal meiotic errors (40), and this concurs with the elevated frequency of sex chromosomal disomy in human sperm.

Effect of Donor Age. With the maternal age effect accepted as an irrefutable phenomenon, attempts have been made to determine whether a paternal age effect exists for aneuploidy. Several epidemiologic studies have been carried out with conflicting results. The different populations investigated by these studies include aneuploid abortuses; liveborns; fetuses which had been diagnosed prenatally; and adults from

endocrine/fertility clinics, mental institutions or hospitals. Unfortunately, problems with sample differences, confounding variables and the inability to isolate maternally derived cases from paternally derived cases render it very difficult to assign a definitive role for paternal age in aneuploidy from these studies. To overcome some of these difficulties, we studied 18 healthy men stratified into six different age groups (20–24, 25–29, 30–34, 35–39, 40–44, and 45 years or older), with three men in each group. Multicolor FISH was performed on sperm from each donor using DNA probes for chromosomes 1, 13, 21, X, and Y. A minimum of 30,000 sperm per donor was analyzed, for a total of 540,000 sperm (41, unpublished results). The only chromosome to show a significant paternal age effect was YY disomy (Pearson correlation coefficient, $r = 0.584$, $P < 0.02$). Other FISH studies have also demonstrated a significant increase in the frequency of sex chromosomal abnormalities with donor age. In a study of 24 men and 400,000 sperm, Griffen *et al.* found that donor age had a significant role in XX, YY, and XY disomy (42). Robbins *et al.* (43) found an age effect for XX and YY disomy frequencies in 200,000 sperm from 14 men. In a previous study (44), our laboratory found evidence for an age effect for YY disomy and disomy 1 in 10 men. From these studies it does appear that the sex chromosomes, in particular, may demonstrate an increased frequency of nondisjunction with paternal age.

Men at Potential Increased Risk

Chemotherapy Patients. Testicular cancer is common among young men, and many affected men are concerned about the potential of genetic damage after CT. However, there is very little information available about the risks of genetic damage in human sperm and the possibility of passing on abnormalities to future offspring (45).

Studies of sperm karyotypes in men treated with CT have been confusing as some have demonstrated a significant increase in the frequency of chromosomal abnormalities (46–48) after treatment, whereas others have not (17,49). One difficulty is the sample sizes possible with the human sperm/hamster oocyte technique as well as the great variability in types of cancer and treatment regimens studied. We have attempted to overcome some of these problems by studying the frequency of sperm chromosomal abnormalities in cancer patients with testicular tumors before and after the same CT treatment. FISH analysis with chromosome-specific DNA probes was used to allow analysis of a

large sample of sperm in each patient as well as sperm karyotype analysis to provide precise information about both structural and numerical chromosomal abnormalities.

We have studied four patients with testicular cancer before and 2 to 13 years after CT with BEP (50). Using the human sperm–hamster oocyte system, a total of 788 sperm chromosome complements was studied, 236 before CT and 552 after CT. There was no significant difference in the total frequency of sperm chromosomal abnormalities pre-CT (10.2%) compared to post-CT (10.7%). Similarly, there were no significant differences in the frequencies of numerical abnormalities (2.5% pre-CT vs 2.4% post-CT) or structural abnormalities (6.4% pre-CT vs 7.4% post-CT). The percentage of X-bearing sperm was also not significantly different before (46.3%) and after CT (50.1%). The results in cancer patients were not significantly different from control donors. This study corroborates results from our previous analysis of these same men using multicolor FISH for assessment of aneuploidy for chromosomes 1, 12, X, Y, and XY (51). A total of 161,097 sperm was analyzed, 80,445 before and 80,642 after treatment. The mean disomy frequencies pre-CT and post-CT were 0.11 and 0.06% for chromosome 1, 0.18 and 0.15% for chromosome 12, 0.10 and 0.09% for the X chromosome, 0.13 and 0.10% for the Y chromosome, and 0.25 and 0.20% for XY sperm, respectively. There was no significant difference in the frequency of disomy pre-CT vs post-CT for any chromosome, except chromosome 1 demonstrated a significant decrease after CT. The “sex ratios” and frequency of diploid sperm were also not significantly different in pre- and post-CT samples with 50.2% X-bearing sperm pre-CT and 50.5% X post-CT and 0.14% diploid sperm pre-CT vs 0.15% diploid sperm post-CT. None of the values in the cancer patients differed significantly from those in 10 normal control donors. Together, these two types of analyses suggest that men treated with BEP CT do not have an increased risk of chromosomal abnormalities in their sperm 2 or more years after treatment.

Infertility Patients. There is some concern that infertile men may have a higher frequency of chromosomal abnormalities in their sperm and that intracytoplasmic sperm injection (ICSI) may bypass normal selection barriers, leading to a higher frequency of chromosomally abnormal offspring. Recent reports based on prenatal diagnosis in ICSI pregnancies have indicated an increased risk of sex chromosomal abnormalities of approximately 1% (52). In a study of five infertile men with oligospermia, asthenospermia, or teratospermia,

we demonstrated an increased frequency of numerical chromosomal abnormalities in sperm of infertile men by two different techniques: the human sperm–hamster oocyte fusion system, which allows analysis of human sperm karyotypes, and multicolor FISH analysis of chromosome-specific DNA probes (53). Approximately 100 sperm karyotypes were analyzed for each infertile patient. The results demonstrated a significant increase in the frequency of total sperm chromosome abnormalities in infertile patients compared to control donors ($P < 0.005$). Multicolor FISH analysis was performed on a minimum of 10,000 sperm nuclei/chromosome probe/patient for chromosomes 1, 12, X, and Y. There was a significant increase in the frequency of disomy for chromosome 1 ($P < 0.005$) and particularly for XY disomy ($P < 0.001$) in infertile patients. If a sperm with disomy 1 fertilized a normal oocyte, a trisomy 1 embryo would be produced, which would likely be lost before implantation because of the lethality of this condition. However, a 24,XY sperm would produce a 47,XXY embryo with Klinefelter syndrome, which has a high probability of surviving to term.

This study was expanded to analyze sperm chromosomal abnormalities in more infertile men (9) and to test the frequency of aneuploidy in sperm for chromosomes 13 and 21 because trisomies for these chromosomes cause clinically significant chromosome syndromes which survive to term (Patau syndrome and Down syndrome, respectively). A total of 306,035 sperm was analyzed in the infertile patients. There was a highly significant increase in the frequency of disomy for chromosomes 1 ($P < 0.005$), 13 ($P < 0.001$), and 21 ($P < 0.003$) and XY disomy ($P < 0.001$) in infertile patients compared to control donors. Results in this expanded series of infertile patients have corroborated our preliminary results in five patients. Namely, there is an increased frequency of sperm with disomy for chromosome 1 and XY disomy in infertile patients compared to normal control donors. We have also demonstrated a highly significant increase in the frequency of disomy for chromosomes 13 and 21.

Pang *et al.* (54) studied sperm aneuploidy by FISH analysis of chromosomes 7, 11, 12, 18, X, and Y in nine patients with oligoasthenoteratozoospermia and also found an increased frequency of numerical chromosomal abnormalities, including sex chromosomal aneuploidy, in the infertile men. Miharu *et al.* (55) found no significant difference in the frequency of aneuploidy as assessed by one-color FISH in spermatozoa from 12 infertile men compared to normal donors; however, a relatively small number of sperm nuclei

were studied per male and XY aneuploidy could not be evaluated because they did not use multicolor FISH.

Our results from both FISH analysis and sperm karyotypes demonstrated a significantly increased frequency of aneuploidy in sperm from infertile men. These results were most striking in men with oligospermia. Quantitative problems, including oligozoospermia and azoospermia, have been associated with pairing abnormalities within both the autosomes and the sex chromosomes. Egozcue *et al.* (56) reported that in infertile males, there is an increased frequency of pairing disruptions resulting in meiotic arrest. It is possible that a pairing abnormality in these infertile males could lead to meiotic arrest in some cells, causing oligozoospermia and aneuploidy in other cells capable of completing spermatogenesis. The sex chromosome bivalent is particularly susceptible to pairing abnormalities because there is generally only one crossover in the pseudoautosomal region. Speed and Chandley (57) studied infertile men with a normal somatic karyotype and found reduced XY synapsis compared to normal men. Furthermore, Hassold *et al.* (58) have determined that there is a reduction in recombination for the XY bivalent in meiosis leading to a 47,XXY karyotype. Thus it is quite plausible that infertile men have a decreased frequency of recombination and pairing, leading to both meiotic arrest (oligospermia) and nondisjunction of chromosomes causing aneuploidy.

It is interesting that the XY bivalent has the highest frequency of aneuploidy observed in sperm from infertile patients. The fact that sex chromosomal abnormalities occur at an increased frequency in pregnancies after ICSI (similar to the frequency in sperm of infertile patients) suggests that these sex chromosomal aneuploidies are not selected against.

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