THE UNIVERSITY OF CALGARY

MEIOTIC SEGREGATION OF SPERM CHROMOSOMES IN TWO HUMAN RECIPROCAL TRANSLOCATION HETEROZYGOTES

by

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A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE

DEGREE OF MASTER OF SCIENCE

DEPARTMENT OF MEDICAL SCIENCE

CALGARY, ALBERTA

DECEMBER, 1990

ISBN 0-315-67001-0

THE UNIVERSITY OF CALGARY

FACULTY OF GRADUATE STUDIES

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled, "Meiotic Segregation of Sperm Chromosomes in Two Human Reciprocal Translocation Heterozygotes" submitted by Elizabeth L. Spriggs in partial fulfillment of the requirements for the degree of Master of Science.

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December 19, 1990

ABSTRACT

Individuals heterozygous for reciprocal translocations are at an increased risk for reproductive failure and chromosomally abnormal progeny. Studies of liveborn offspring or fetuses do not provide accurate information about meiotic segregations, as lethal segregations have already been lost through spontaneous abortions. In contrast, direct analysis of sperm chromosome complements more accurately reveals the proportion of gametes that are chromosomally unbalanced.

Using the hamster oocyte/human sperm fusion technique, meiotic segregation was studied in two male reciprocal translocation heterozygotes, 46,XY,t(11;17)(p11.2;q12.3) and 46,XY,t(1;11)(p36.3;q13.1). For the t(11;17) heterozygote, 202 sperm chromosome complements were obtained, of which 18 karyotypes were not included in the segregation data because of multiple breaks and rearrangements. The frequency of chromosomally unbalanced sperm complements related to the translocation was 61.4%. There were 6.4% numerical, 19.3% structural and 0.5% numerical and structural abnormalities unrelated to the translocation. For the t(1;11) carrier, a total of 575 sperm chromosome complements was obtained, with 27 spreads not included in the segregation data due to the presence of multiple breaks and rearrangements. The frequency of chromosomally unbalanced sperm related to the translocation was 66.8%. Unrelated numerical and

structural abnormalities accounted for 5.6% and 9.2%, respectively of the karyotypes. All types of segregations, with the exception of 4:0 segregation, were observed in the sperm of the t(11;17) and t(1;11) carriers. Alternate segregation, where a 1:1 ratio of normal to balanced karyotypes was observed, is the only segregation resulting in chromosomally balanced sperm. Adjacent I segregation produced the majority of chromosomally unbalanced sperm, followed by adjacent II and then 3:1 segregations. For both heterozygotes studied, the ratio of X- and Y-bearing sperm was not significantly different from the expected ratio of 1:1.

The frequencies of numerical abnormalities obtained in these two reciprocal translocation carriers were compared to the frequency of aneuploidy observed in the normal population to determine if an interchromosomal effect was present. Although the sample sizes were large enough to detect a tripling and a doubling in the aneuploidy frequency for the t(11;17) and t(1;11) carriers, respectively, neither displayed an increased frequency in the conservative estimate of numerical abnormalities. Hence, results obtained from sperm chromosome analysis for these reciprocal translocation carriers do not support the concept of an interchromosomal effect.

Acknowledgements

First and foremost, I would like to thank my supervisor, Dr. Renée Martin, for her continual guidance and encouragement throughout my M.Sc. program. Members of my supervisory committee, Dr. David Cox and Dr. Gil Schultz, are gratefully acknowledged for their suggestions and advice. I thank Dr. Michael Hines for serving on my examination committee.

Special thanks to Dr. Maj Hulten for ascertaining the two reciprocal translocation carriers and sending samples to Calgary.

Many thanks to all members of Renée's laboratory, especially Leona, Kathy, and Evelyn for teaching me all they knew about the human sperm/hamster fusion technique. My sincere thanks to Evelyn for careful reading of this manuscript.

The financial support of Alberta Heritage Foundation for Medical Research is gratefully acknowledged.

Finally, special thanks to all my family, particularly Bob, my parents, my mother-in-law, Marlene, and Jim. Without their support, this thesis would not have been possible.

Dedication

To my parents and my husband who always believed in me.

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LIST OF ABBREVIATIONS

ace	acentric fragment
adjI	adjacent I segregation
adjII	adjacent II segregation
asy	asymmetrical exchange
BWW	Biggers-Whitten-Whittingham medium
°C	degree Celsius
cen	centromere
csb	chromosome break
csg	chromosome gap
ctb	chromatid break
cte	chromatid exchange
ctg	chromatid gap
del	deletion
der	derivative
dic	dicentric
G	force of gravity
g	gram
HAL	haploid autosomal length
inv	inversion
ISCN	International System for Human Cytogenetic Nomenclature
IU	International Units
IVF	*in vitro* fertilization
M	molarity
mar	marker
MB	multiple breaks
MB+R	multiple breaks and rearrangements
mg	milligram
MII	metaphase II
ml	millilitre
mm	millimetre
N	normality
p	short arm of a chromosome
q	long arm of a chromosome
Q-band	quinacrine dihydrochloride banding stain
qr	quadriradial
rob	Robertsonian translocation
sym	symmetrical exchange
t	translocation
ter	terminal end of a chromosome
TEST	TES-Tris
tr	triradial
ug	microgram
ul	microlitre
um	micrometre
X^2	chi square statistic

I. INTRODUCTION

Reciprocal translocations, the exchange of terminal segments between non-homologous chromosomes, have been identified in a variety of species including man (Ford and Clegg, 1969). From studies of consecutive live births and prenatal diagnoses where chromosome banding was performed, the incidence of reciprocal translocation carriers was determined to be 1 in 625 (Van Dyke et al., 1983). Approximately 25% of the reciprocal translocations detected in the above amniocentesis study were de novo and the remaining 75% of reciprocal translocations were inherited (Van Dyke et al., 1983). Provided no genetic material has been lost or disturbed, these individuals are phenotypically normal. The only hidden difference is their increased risk for reproductive failure and chromosomally unbalanced progeny.

A. Reproductive Consequences

In the germ cells of reciprocal translocation carriers, the two translocated and the two corresponding normal chromosomes must pair, resulting in the formation of a quadrivalent. Because this quadrivalent can segregate in several ways, a proportion of gametes is chromosomally unbalanced, causing either recognized or unrecognized spontaneous abortions or chromosomally abnormal progeny. However, for some reciprocal translocation carriers, the presence of such structural rearrangements disturbs gametogenesis and reduces the

number of germ cells produced, resulting in decreased fertility (de Boer, 1986, Chandley, 1988). Hence, most individuals heterozygous for reciprocal translocations are ascertained through an unexpected reduction in fertility or through the birth of a chromosomally unbalanced child (Ford and Clegg, 1969).

i. Types of segregations

From the quadrivalent, a total of sixteen different gametes are theoretically possible, but only two of these are chromosomally balanced. Most commonly observed is 2:2 segregation, where two chromosomes move to each pole. Less frequent is 3:1 segregation, where three chromosomes separate from the remaining chromosome, and 4:0 segregation where all four chromosomes move to one pole.

a. 2:2 segregation

The 2:2 segregation can occur in three ways, producing six different gametes (See Figure 1). In alternate segregation, the only segregation that will result in chromosomally balanced progeny, half of the gametes will carry both normal chromosomes and the other half will carry both translocated chromosomes. In adjacent I and adjacent II segregations, chromosomes with homologous centromeres move to opposite or the same poles, respectively, at anaphase I. Adjacent segregations produce chromosomally unbalanced gametes containing various duplications and deficiencies.

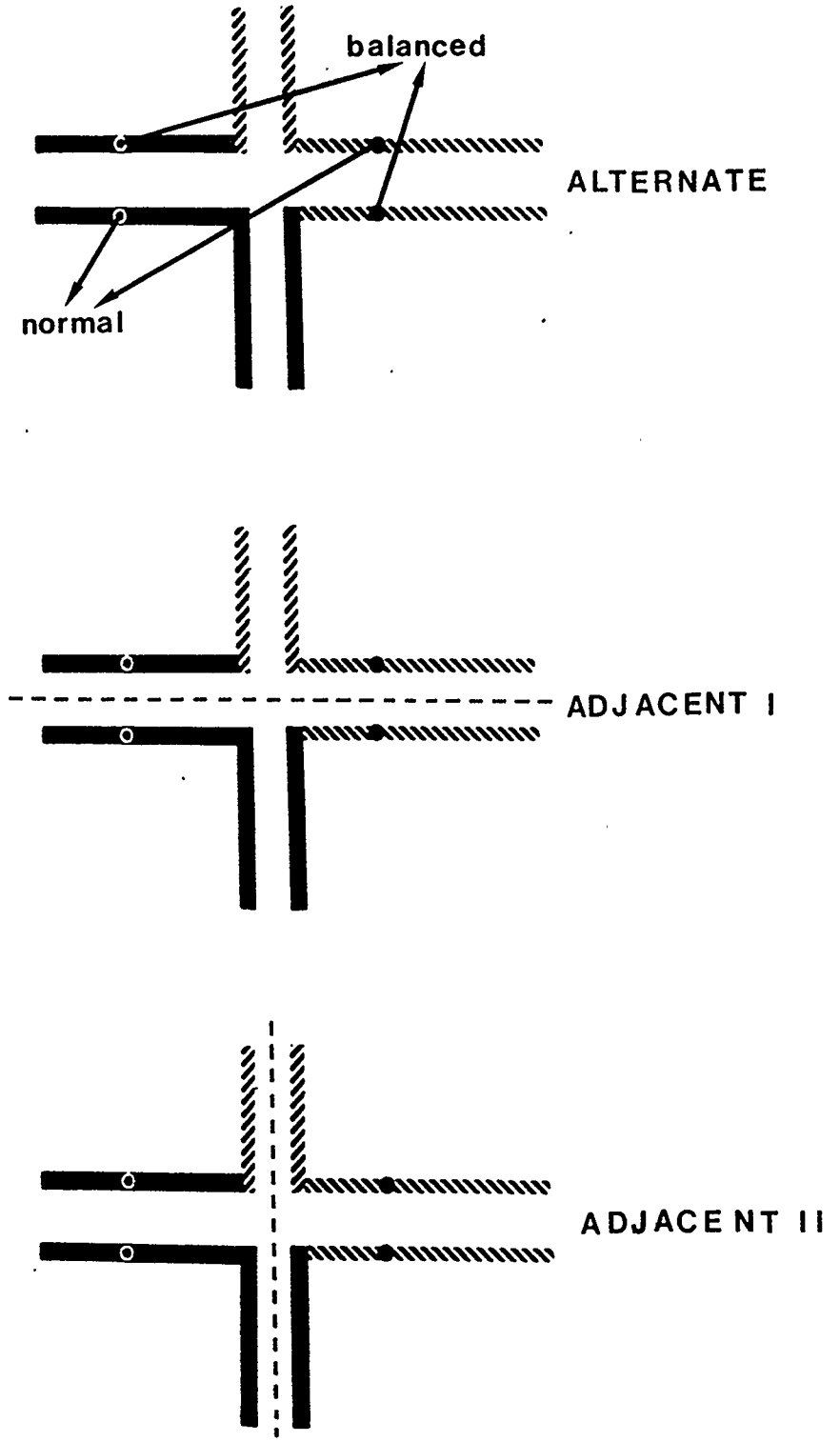

Figure 1: Types of 2:2 segregation.

b. 3:1 segregation

3:1 segregation produces eight different gametes that are either partially disomic or partially nullisomic. Resulting offspring, if the chromosomal content is compatible with life, will have 45 or 47 chromosomes. Two types of 3:1 segregation have been defined (Jalbert and Sele, 1979, Lindenbaum and Bobrow, 1975). First, segregation of two normal chromosomes with one of the translocated chromosomes results in tertiary trisomy and its complement, the other translocated chromosome, results in tertiary monosomy. Secondly, segregation of the two translocated chromosomes with one normal chromosome results in interchange trisomy and its complement, the other normal chromosome, results in interchange monosomy.

c. 4:0 segregation

In 4:0 segregation, all four chromosomes of the quadrivalent segregate to one pole and none travel to the other pole. This type of segregation has never been observed in any clinically recognized pregnancy but it has been observed in one human sperm (Burns et al., 1986).

ii. Chromosomally unbalanced progeny

Individuals heterozygous for reciprocal translocations have a risk of approximately 6% for producing chromosomally unbalanced liveborn offspring (Davis et al., 1985). However, if the couple has been ascertained through the birth of a chromosomally abnormal child, the recurrence risk is greater than 6%; if

ascertained with a history of repeated spontaneous abortions, the risk is less than 6% (Davis et al., 1985). Such offspring are usually the result of the segregation that produces the least imbalance (Lindenbaum and Bobrow, 1975, Jalbert et al., 1980). The maximum imbalance that allows intrauterine survival to term is approximately 4% trisomy and 2% monosomy of the haploid autosomal complement (Daniel, 1979), with a mean imbalance of approximately 1.4% trisomy and 0.2% monosomy of the haploid autosomal complement (Davis et al., 1985). Different chromosomes show different degrees of tolerance for chromosomal imbalance. For example, imbalances involving chromosomes 9, 21, and 22 occur more frequently and imbalances involving chromosomes 1, 2, 3, 6, 8, 17, and 19 are seen less frequently (Davis et al., 1985).

The segregations responsible for the production of chromosomally unbalanced children, in order of decreasing frequency are adjacent I, 3:1 and adjacent II (Jalbert and Sele, 1979). While adjacent I segregation was evenly distributed in chromosomally unbalanced children from maternal and paternal carriers, 3:1 and adjacent II disjunctions were largely found to be maternally derived (Lindenbaum and Bobrow, 1975, Jalbert and Sele, 1979, Daniel et al., 1989). Amniocentesis data showed that both maternal and paternal carriers of reciprocal translocations had equal frequencies of chromosomally unbalanced fetuses (Boue and Gallano, 1984).

As mentioned earlier, there are four possible types of gametes that can arise from 3:1 segregation. Of these four, tertiary trisomy is most commonly found in liveborn offspring, whereas interchange monosomy is never found in liveborn offspring (Gardner and Sutherland, 1989). As expected in chromosomally unbalanced liveborns resulting from tertiary and interchange trisomy, the extra chromosome observed is the shorter one, which keeps extra genetic material to a minimum. On the other hand, the longer translocated chromosome is always present in tertiary monosomic children, thus limiting the amount of genetic material missing. (Lindenbaum and Bobrow, 1975).

Using the observation that the segregations with the minimal imbalance are found in chromosomally unbalanced progeny, it is possible to predict which segregations are compatible with the viability of such pregnancies. Jalbert et al. (1980) developed rules, based on the pachytene diagram, for predicting the most probable segregation(s) that would produce chromosomally abnormal liveborns. Likewise, a computer program has been developed to generate all unbalanced gametes from adjacent I, adjacent II and 3:1 segregations and to calculate the corresponding percentage of imbalance (De Arce et al., 1986). The least imbalanced gamete is then selected as the most probable segregation to be detected in chromosomally abnormal children or miscarriages. Both of these methods are 87% accurate (De Arce et al., 1986).

Errors are probably due to the fact that the size of imbalance is only one determinant in the outcome of an unbalanced pregnancy. Chromosomal content of the segment involved is another determinant that is just beginning to be investigated (De Arce et al., 1986).

iii. Infertility

In infertility clinics, there is a ten-fold increase in the number of males with reciprocal or Robertsonian translocations over the general population (Gabriel-Robez et al., 1986). For reasons not fully understood, the presence of a structural rearrangement disrupts gametogenesis in some men, resulting in oligo- or azoospermia (Chandley, 1988). Studies on female mice heterozygous for reciprocal translocations revealed a reduction in ovarian volume and oocyte numbers when compared to their normal counterparts (Mittwoch et al., 1981, Setterfield et al., 1988).

As a consequence of synapsis, where each segment of the rearranged chromosomes attempts to pair with the homologous segment of the normal unchanged chromosomes, a cross-shaped figure is formed (Ford and Clegg, 1969, Gardner and Sutherland, 1989). If chiasmata are formed in all or three arms of the cross, a ring quadrivalent or a chain of four chromosomes, respectively, will follow (Ford and Clegg, 1969, Swanson et al., 1981). With the failure of chiasmata to form in two or more arms, the quadrivalent will separate into a trivalent and a univalent, two

bivalents, a bivalent and two univalents, or even four univalents (Ford and Clegg, 1969). In human male reciprocal translocation carriers, all possible configurations have been observed. However, in fertile males carrying a reciprocal autosomal translocation, ring quadrivalents were predominately observed; in males heterozygous for a reciprocal autosomal translocation with oligo- or azoospermia, there was a preponderance of chain configurations (Chandley, 1988, Templado et al., 1990).

Reciprocal translocations carried by infertile males tend to be characterized by one breakpoint near the end of the chromosome (de Boer, 1986, Chandley, 1988). With the presence of a short pairing segment, crossing-over may fail to occur, resulting in chain configurations. Gabriel-Robez et al. (1986) observed a significant relationship between the presence of an acrocentric chromosome in the translocation and male sterility. In such cases, chain configurations would tend to be observed since crossing-over rarely occurs in the short arm of acrocentric chromosomes (Chandley, 1988). How the presence of some structural rearrangements causes germ cell death is not clear; several hypotheses have been proposed. However, it is generally believed that a lack of complete meiotic pairing in such translocation carriers is somehow associated with the degeneration of germ cells (Chandley, 1988). Several studies have been conducted on mice heterozygous for reciprocal translocations. To date,

these studies have shown fertility of reciprocal translocation carriers to be dependent on the genetic background (de Boer, 1986).

It is difficult to detect a similar reduction in fertility for female heterozygotes, because of the vast number of oocytes potentially available. Studies in mice have revealed that female reciprocal translocation carriers have reduced ovary size (Mittwoch et al., 1981) and lower numbers of oocytes (Setterfield et al., 1988). It is difficult to study these variables in man, but a reduction in the reproductive life span or early menopause may indicate reduced fertility (Chandley, 1988). To date, there have been some reports of early menopause in female carriers of X-autosome translocations (Chandley, 1988).

B. Animal Studies

The segregation pattern of such translocated chromosomes has been studied extensively in plants and insects including maize, rye, onion fly, cabbage loopers, and fruit fly (Vosselman, 1981, North, 1978). In contrast, information available on mammalian reciprocal translocation carriers is minimal (de Boer, 1976). The meiotic products of reciprocal translocation heterozygotes have been studied only in the mouse, the pig and the hamster. Generally, the data from earlier studies is of poor quality as most authors were unable to distinguish between balanced and unbalanced gametes, whereas later studies on hamsters provided a wealth of information.

Oshimura and Takagi (1975) studied the frequency of 3:1 disjunction in the mouse reciprocal translocation T(14;15)6Ca by analyzing metaphase II (MII) cells and embryos obtained from a back cross. They found an increased incidence of trisomies in the progeny of female carriers compared to that found in the progeny of male carriers, and a higher frequency of 3:1 segregations in oocytes (22.2%) than in spermatocytes (4.4%). Due to unfavorable morphology of MII chromosomes, they were unable to accurately score the incidences of the various 2:2 segregations.

Using two different reciprocal mouse translocations, T(2;8)26H and T(1;13)70H, de Boer (1976) studied the chromosomes in metaphase I and II cells and compared fertility in translocation heterozygotes to fertility in normal mice. Again, not all normal and translocated chromosomes could be positively identified. Nonetheless, he was able to estimate from metaphase II spermatocytes that the frequency of adjacent II segregations was 8.5% and 25.2% for the T26H and T70H heterozygotes, respectively. From metaphase I spermatocytes, he was able to estimate the frequency of 3:1 segregations for T26H and T70H carriers to be virtually 0% and 4-5%, respectively. From the relative fertility scores, he again estimated the frequencies of adjacent II and 3:1 segregations as 9.8% and 29.0% for both male heterozygotes and 9.4% and 27.8% for both female carriers.

Akesson and Henricson (1972) karyotyped 113 embryos obtained from mating a boar heterozygous for the reciprocal translocation, (4q-;14q+), with several normal sows. Twelve of the 113 (11%) embryos were chromosomally abnormal with the majority arising from adjacent I and II segregations. However, this boar had a reduction in fertility of 34%, three times the frequency of the abnormal embryos. The authors were unable to explain this difference, but it is now assumed that certain unbalanced gametes result in immediate, undetected loss upon fertilization (Plachot et al., 1987).

King et al. (1981) studied the chromosomal constitution of embryos produced by crossing pigs heterozygous for the reciprocal translocation, (13q-; 14q+), with either normal pigs or pigs heterozygous for the same reciprocal translocation. Karyotyping these embryos revealed some unbalanced segregations, particularly adjacent I and II segregations, in the preimplantation stage and no unbalanced segregations in the postimplantation stage. Using the frequency of living embryos and the numbers of corpora lutea, embryonic mortality was predicted to be 72.3% for heterozygous gilts crossed with normal boars, 68% for heterozygous boars mated with normal gilts, and 85.5% for heterozygous gilts crossed with heterozygous boars.

Using male Chinese hamsters heterozygous for one of four different reciprocal translocations, Sonta (1984)

studied the chromosomes from metaphase II cells. The frequency of cells with an unbalanced karyotype ranged from 54.3% to 60.4%. Adjacent II and 3:1 segregations were responsible, respectively, for 6.3% to 18.5% and 4.2% to 19.6% of the chromosomally unbalanced cells. Thus, Sonta (1984) concluded that different reciprocal translocations produced different segregation ratios.

Sonta and Kitayama (1987) did a further study using male Chinese hamsters heterozygous for one of eight different reciprocal translocations to determine if there was a correlation between certain characteristics of the translocations and the segregation ratios. Here, adjacent I, adjacent II, and 3:1 segregations were responsible, respectively, for 27.3% to 36.0%, 6.0% to 19.4%, and 4.1% to 22.0% of the metaphase II cells. While no specific characteristics appeared to be related to the frequency of adjacent II segregation, they did find that the length of the chromosomes involved in the translocation was related to the frequency of 3:1 segregation.

As seen above, information available on the early studies of mammalian reciprocal translocation heterozygotes is indeed scanty. Data obtained on spermatocytes and oocytes was often incomplete because of the poor chromosome morphology, and data obtained from embryos did not include those gametes that would result in early embryonic loss. However, recent studies on the heterozygosity of reciprocal translocations in Chinese

hamsters has provided more accurate and detailed data. Although these studies on plants and animals provide information on the meiotic segregations and clues about the behaviour of quadrivalents during meiosis, it is difficult to extrapolate the specific parameters, such as frequencies of unbalanced gametes, to human reciprocal translocation carriers.

C. Human Studies

Prior to the development of the human sperm/hamster oocyte fusion technique, knowledge on human reciprocal translocation carriers was limited to meiotic studies, family histories, and data from prenatal diagnoses. None of these sources was able to provide accurate information about meiotic segregations.

As mentioned earlier, meiotic studies of human spermatocytes provide information about the meiotic pairing behaviour of reciprocally translocated chromosomes. All possible configurations have been observed: ring quadrivalents, chain quadrivalents, trivalents, bivalents, and univalents (Chandley, 1988, Templado et al., 1990). However, the relationship between the various meiotic configurations and the actual meiotic segregations is not clear. While the trivalent/univalent combination increases the likelihood of 3:1 segregation, it is not the only configuration to result in 3:1 disjunction, as both ring and chain quadrivalents may undergo 3:1 segregation. (Lindenbaum and Bobrow, 1975).

In addition, no association between the configurations observed and the frequency of the various 2:2 segregations could be detected (de Boer, 1976). Thus, studies of meiotic pairing in human spermatocytes cannot be used to determine the frequency of chromosomally unbalanced gametes.

It is difficult to estimate the risk of a chromosomally unbalanced liveborn offspring from families referred to genetic clinics because of the severe ascertainment bias. Families with several affected children are more likely to be ascertained than those without abnormal children. Also, the study of liveborn children does not provide accurate information about the meiotic segregations because lethal segregations have already been lost to recognized or unrecognized spontaneous abortions (Martin et al., 1990a).

Through amniocentesis, Boue and Gallano (1984) studied the karyotypes of fetuses where one parent was a known heterozygote for a structural rearrangement. This provided a more objective method to estimate the risk of a chromosomally abnormal liveborn child. For reciprocal translocations, 12% of the fetuses were chromosomally unbalanced and this incidence was not related to the sex of the carrier. However, chromosomally abnormal fetuses detected during amniocentesis are more likely to survive to term as most chromosomally unbalanced conceptuses are lost during the first trimester of pregnancy. Again, data

from prenatal diagnosis does not provide accurate information about the meiotic segregations because certain segregations have already been lost through spontaneous abortions.

To circumvent the problem of losing lethal segregations to early spontaneous abortions, the chromosomal constitution of human gametes must be directly examined. With the development of the human sperm/hamster oocyte technique, this became possible (Rudak et al., 1978).

D. Interchromosomal Effect

It has been proposed that abnormal pairing configurations found in individuals heterozygous for balanced structural rearrangements interfere with the normal disjunction of chromosomes (Warburton, 1985). The term "interchromosomal effect" has been coined for such associations between aneuploidy and structural rearrangements. While this effect has been demonstrated in some organisms, especially in Drosophila, there is still some controversy as to whether an interchromosomal effect is really present in human reciprocal translocation carriers.

i. Evidence in Drosophila

Using a female Drosophila trisomic for chromosome 4 and part of the X chromosome, Grell (1971) was able to demonstrate that the two heterologues, the 4 and the deleted X chromosomes, segregated to opposite poles 99% of

the time. However, the results obtained could not be explained by homologous chromosome pairing followed by independent assortment.

Based on the above studies, Grell (1971) proposed that two pairing events, not one, occur during meiosis (See Figure 2). In the first or exchange pairing, chromosome pairing is based solely on homology. If crossing-over fails to occur between homologous chromosomes, these chromosomes then enter a distributive pool and are given a second chance to pair. This second form of pairing, called distributive pairing, is solely dependent upon chromosome size. The size criterion should still favour the pairing of homologues over the pairing of heterologues and thus, normal gametes should still be obtained. The main difference between exchange pairing and distributive pairing is that crossovers cannot occur in distributive pairing.

Pairing between heterologues may be favoured only in individuals who are either aneuploid for all or part of a chromosome or are heterozygous for structural rearrangements. The presence of an extra or rearranged chromosome may disturb the segregation of a similarly-sized pair of homologous chromosomes. For reciprocal translocation carriers, a quadrivalent, instead of the usual pair of bivalents, is formed during meiosis. This increases the likelihood for a noncrossover chromosome, particularly for those translocations that are extremely

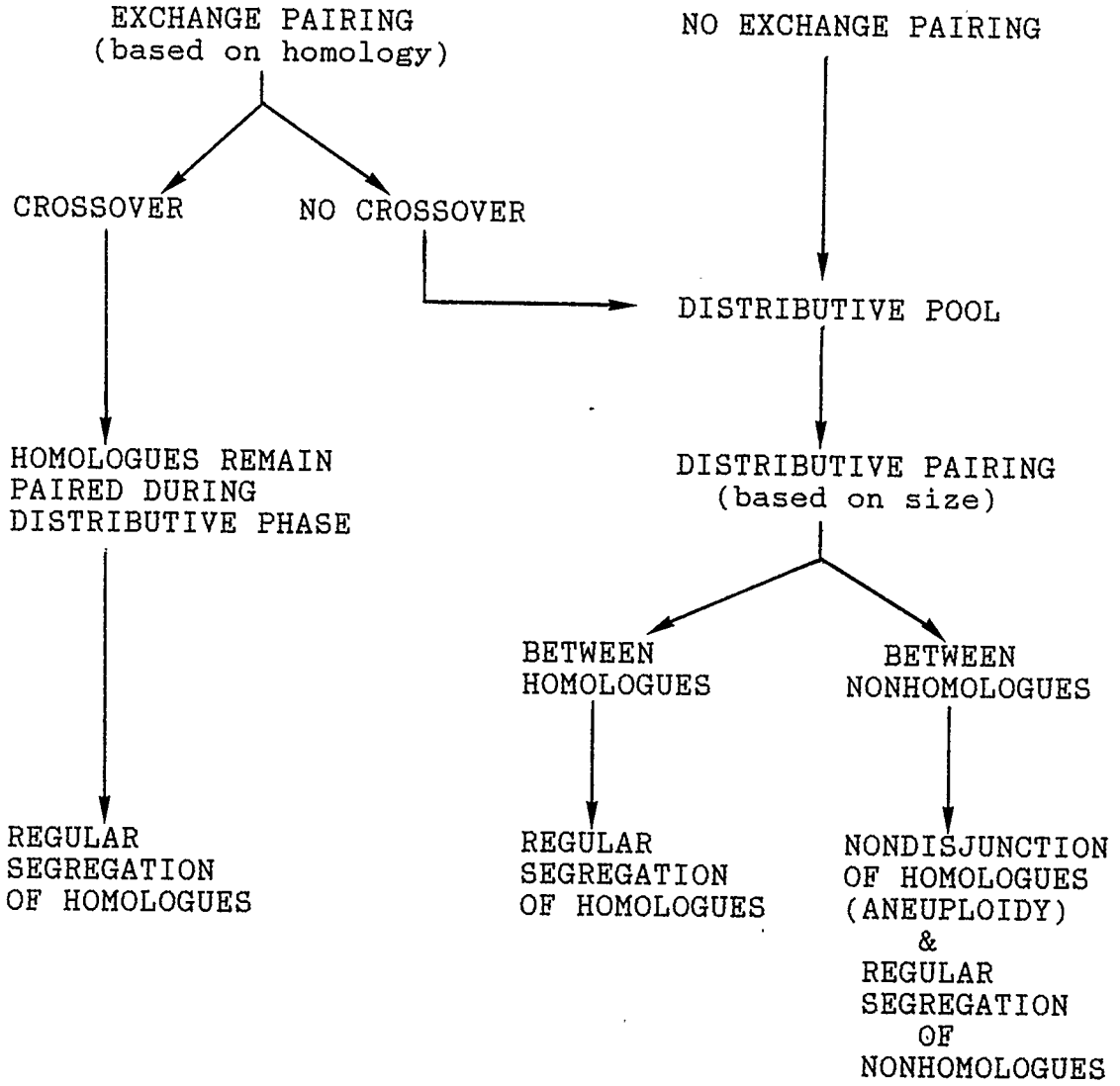

Figure 2: Proposed meiotic pairing scheme based on Grell's hypothesis of distributive pairing (From Grell, 1971).

asymmetric, where the probability of a crossover occurring in the small chromosome is drastically reduced. This small noncrossover chromosome may then disrupt the pairing of a similarly-sized pair of homologous chromosomes. Non-disjunction, resulting from heterologous pairing, is termed distributive non-disjunction. (Grell, 1971)

Although it is clear that chromosomal aberrations in Drosophila display an interchromosomal effect (Grell, 1971), the connection between aneuploidy and structural rearrangement in man is not as obvious.

ii. Evidence in man

Lejeune (1963) was the first to observe the presence of both aneuploidy and structural rearrangements within the same family. Over the next twenty years, evidence for an interchromosomal effect in man was largely based on single cases or small series where the possibility of a coincidence or bias could not be ruled out (Aurias et al., 1978, Jacobs, 1979, Lindenbaum et al., 1985). In 1985, studies with substantial sample sizes were conducted (Lindenbaum et al., 1985, Warburton, 1985). However, results from these and subsequent studies provide evidence both for and against the theory of an interchromosomal effect. Hence, it has not been satisfactorily concluded whether an interchromosomal effect is present or absent in man.

In the first large-scale study, Lindenbaum et al. (1985) found a higher incidence of balanced structural

rearrangements than expected in the parents of 1454 children with regular trisomy 21. Of the 945 parents karyotyped, 1.06% had reciprocal translocations and 0.11% had Robertsonian translocations, representing 0.34% reciprocal translocations and 0.03% Robertsonian translocations amongst all parents, tested and untested (2908), in the study. The frequencies for reciprocal translocations exceeded that observed in consecutively-banded newborn infants (0.16%) and the frequencies for Robertsonian translocation did not exceed the rate observed in the newborn studies (0.11%).

Likewise, Couzin et al. (1987) did a retrospective cytogenetic study on the parents of 96 children with primary trisomy 21 Down's syndrome and found 4 parents carrying structural rearrangement: 2 reciprocal translocations and 2 pericentric inversions. They tentatively suggested that their frequencies also supported the contention of an interchromosomal effect.

However, Warburton (1985) argued that results obtained by karyotyping parents of children with trisomy 21 are not valid because the approach was flawed. Instead of examining the probability of children with trisomy 21 having a parent with a structural rearrangement, the theory of an interchromosomal effect should be studied by examining the probability of a structural rearrangement resulting in a trisomic child. Warburton (1985) analyzed the data obtained from amniocentesis in 1300 pregnancies

studied because a parent was known to be a balanced translocation carrier. There was no apparent excess of trisomies present in these pregnancies. Warburton (1985) also examined the question of an interchromosomal effect by asking whether the frequency of inherited translocations is greater than expected in trisomic newborns. Data from this smaller study did not suggested any evidence of such an effect.

Finally, Uchida and Freeman (1986) demonstrated that in three informative families, the parent who transmitted the extra chromosome 21 was not the one with the structural rearrangement. Thus, the presence of a structural rearrangement and trisomy 21 within the same family was purely coincidental and these observations do not lend support to the theory of an interchromosomal effect.

Direct examination of the chromosomal constitution of human gametes could provide further evidence for or against the theory of an interchromosomal effect. However, a sufficient number of sperm karyotypes need to be analyzed to properly test for an increase in the aneuploidy frequency. Based on the frequency of aneuploidy observed in sperm of normal men (2.4%), Martin (1989) has calculated that approximately 160 or 500 sperm karyotypes need to be analyzed to detect a tripling or a doubling, respectively, in the aneuploidy rate.

E. Human Sperm Chromosomes Studies

Human sperm chromosomes cannot be visualized until after fertilization when the pronuclear chromosomes develop (Rudak et al., 1978). In 1976, Yanagimachi et al. discovered that capacitated human sperm could fertilize zona-free golden hamster ova. Rudak et al. (1978) advanced this technique one step to obtain human sperm chromosomes from the male pronucleus in the fertilized hamster egg. With Q-banding, they were able to identify the chromosomes with the same precision as those from somatic cells. In 1982, this technique was successfully duplicated by Martin and her laboratory (Martin et al., 1982) and is now used in 11 different laboratories in the world (Martin, 1989).

In the past seven years, human sperm chromosome complements from twenty men, heterozygous for 21 different reciprocal translocations, have been analyzed (Balkan and Martin, 1983a, Martin, 1984, Burns et al., 1986, Brandriff et al., 1986a, Martin, 1988a, Templado et al., 1988, Pellestor et al., 1989, Templado et al., 1990, Martin et al., 1990a, Martin et al., 1990b, Martin, personal communication). All possible segregations have been observed in human sperm. The frequency of chromosomally unbalanced sperm related to the translocation ranged from 19% to 77%, with a mean of 52% (See Table 1). Adjacent I segregation produced the majority of the chromosomally unbalanced sperm (38%), followed by adjacent II

Table 1: Frequency of sperm carrying various unbalanced meiotic segregations for 21 different reciprocal translocations.

Translocation Observed	# Spreads	Segregations observed (%) AdjI	AdjII	3:1	4:0	Total Unbalanced
(11;22)[1]	13	38.5	23.1	15.4	0.0	76.9
(6;14)[2]	19	31.6	0.0	0.0	0.0	31.6
(7;14)[3]	19	31.6	15.8	0.0	0.0	47.4
(5;11)[4b]	23	26.1	0.0	4.3	0.0	30.4
(7;14)[4b]	23	47.8	0.0	17.4	4.3	69.5
(5;18)[2]	32	15.6	0.0	3.1	0.0	18.8
(5;13)[5]	57	21.1	1.7	0.0	0.0	22.8
(4;17)[5]	60	35.0	6.7	1.6	0.0	43.3
(2;5)[6a]	75	31.3	5.3	21.3	0.0	57.9
(9;18)[5]	82	63.4	0.0	2.4	0.0	65.8
(6;7)[5]	84	48.8	0.0	0.0	0.0	48.8
(1;2)[7]	105	41.9	5.7	11.4	0.0	59.0
(12;20)[8]	113	41.6	9.7	1.8	0.0	53.1
(15;22)[9]	147	39.5	22.4	4.1	0.0	66.0
(4;6)[3]	158	51.9	1.9	0.6	0.0	54.4
(9;10)[10]	168	48.2	5.4	5.9	0.0	59.5
(16;19)[9]	172	27.9	31.4	1.2	0.0	60.5
(3;16)[11]	201	41.3	16.4	5.0	0.0	62.7
(2;9)[3]	208	28.4	24.1	4.3	0.0	56.7
(8;15)[11]	226	38.1	21.2	3.5	0.0	62.8
(3;11)[9]	262	45.8	5.7	0.8	0.0	52.3
Mean	107	37.9	9.4	5.0	0.2	52.4

[a]Some alternate and adjacent I segregations could not be distinguished, so half of the unknown segregation percentages was added to both adjacent I segregation and total unbalanced values.
[b]Both translocations are present in one male.
[1]Martin, 1984
[2]Balkan and Martin, 1983a
[3]Martin et al., 1990b
[4]Burns et al., 1986
[5]Pellestor et al., 1989
[6]Templado et al., 1988
[7]Templado et al., 1990
[8]Martin et al., 1990a
[9]Martin, personal communication
[10]Martin, 1988a
[11]Brandriff et al., 1986a

segregation (9%), 3:1 segregation (5%), and very rarely 4:0 segregation (0.2%). For nineteen reciprocal translocation carriers, the frequencies of numerical and structural abnormalities unrelated to the translocation were within the range observed for normal men (See Table 2). One male, who was heterozygous for two different translocations, had an unusually high rate of numerical abnormalities.

When each reciprocal translocation was examined alone, alternate and adjacent I segregations were always observed, with frequencies of 23.1% to 78.1% and 15.6% to 51.9%, respectively. Adjacent II and 3:1 segregations were not observed in every reciprocal translocation studied and their frequencies ranged from 0% to 31% and 0% to 21%, respectively. Finally, 4:0 segregation was rarely observed. Only one sperm in 2,200 karyotypes analyzed was the result of 4:0 segregation.

The frequency of chromosomally unbalanced sperm is higher than the incidence of chromosomally unbalanced progeny observed at term or in studies of amniocenteses (Boue and Gallano, 1984). While only one or occasionally two segregations are observed in liveborns of each reciprocal translocation carrier, the study of the chromosomal content of human sperm has shown that other segregations are produced. Hence, chromosomally unbalanced conceptuses, with large imbalances, must be selectively eliminated.

Table 2: Frequency of unrelated numerical and structural abnormalities observed in 20 men heterozygous for 21 reciprocal translocations.

Translocation Observed	# Spreads	Numerical Observed	Numerical Estimate+	Structural
(11;22)[1]	13	7.6	0.0	7.6
(6;14)[2]	19	0.0	0.0	10.5
(7;14)[3]	19	0.0	0.0	0.0
(5;11)[4a]	23	69.6*	60.9*	13.0
(7;14)[4a]	23	69.6*	60.9*	13.0
(5;18)[2]	32	15.6	12.5	9.4
(5;13)[5]	57	10.5	0.0	0.0
(4;17)[5]	60	13.3	3.3	0.0
(2;5)[6]	75	12.0	8.0	4.0
(9;18)[5]	82	8.5	4.9	2.4
(6;7)[5]	84	8.3	0.0	1.2
(1;2)[7]	105	14.3	7.6	14.3
(12;20)[8]	113	11.5	1.8	6.2
(15;22)[9]	152	3.9	0.0	8.6
(4;6)[3]	164	9.1	2.4	18.9
(9;10)[10]	171	8.2	5.8	15.2
(16;19)[9]	178	6.0	0.0	9.8
(3;16)[11]	201	1.5	1.0	5.6
(2;9)[3]	217	4.3	0.0	21.7
(8;15)[11]	226	2.6	2.6	2.7
(3;11)[9]	268	3.4	0.0	8.2
Normal Range[12]		4.7(±2.9)	2.4	6.2(±6.0)

+This figure represents the conservative estimate of aneuploidy, which is derived by doubling the frequency of hyperhaploidy.
*Significant increase in the frequency of unrelated numerical abnormalities.
[a]Both translocations are present in one male.
[1]Martin, 1984
[2]Balkan and Martin, 1983a
[3]Martin et al., 1990b
[4]Burns et al., 1986
[5]Pellestor et al., 1989
[6]Templado et al., 1988
[7]Templado et al., 1990
[8]Martin et al., 1990a
[9]Martin, personal communication
[10]Martin, 1988a
[11]Brandriff et al., 1986a
[12]Martin et al., 1987

Each reciprocal translocation carrier produces different percentages of the various segregations. It is not known what factors influence the meiotic segregation ratios. Perhaps, with further studies, factors affecting meiotic segregation, such as type of chromosomes involved, location of breakpoints, or pairing behaviour, may be elucidated (Martin, 1989).

In these studies of men heterozygous for reciprocal translocations, the frequency of structural anomalies ranged from 0% to 21.7%, which is the same range observed in sperm of normal men (0% to 23.1%) (Martin et al., 1987). The frequency of numerical abnormalities unrelated to the translocation ranged from 0% to 16% in sperm of single reciprocal translocation carriers, which is not significantly different from the frequency range of 0% to 10% observed in normal men (Martin et al., 1987). Conversely, in the case of double heterozygote, unrelated numerical abnormalities were observed in 70% of the sperm karyotypes analyzed. While this figure is beyond the normal range and suggests an interchromosomal effect, the researchers (Burns et al., 1986) who studied this carrier's sperm chromosomes have never done a comparable study on normal men.

As previously mentioned, approximately 160 or 500 sperm karyotypes are needed to detect a tripling or a doubling, respectively, in the aneuploidy frequency. Only seven reciprocal translocation carriers have had 160 sperm

analyzed and none of these has shown a significant increase in the aneuploidy rate. There have been no studies where 500 sperm chromosome complements from one reciprocal translocation heterozygote have been analyzed. Thus, further studies, with large sample sizes, are needed on sperm chromosomes of reciprocal translocation carriers to determine if an interchromosomal effect is present or absent in these heterozygotes.

In the present study, sperm chromosomes from two additional reciprocal translocation heterozygotes have been analyzed, in order to provide more information about meiotic segregation patterns and the effect of quadrivalents on disjunction of normal chromosomes. An attempt was made to obtain sample sizes of at least 160, and preferably 500, karyotypes to collect evidence for or against the theory of an interchromosomal effect.

II. RESEARCH OBJECTIVES

Human sperm chromosome complements from two males heterozygous for a reciprocal translocation were obtained using the hamster oocyte-human sperm fusion technique. Resulting sperm karyotypes were Q-banded and analyzed to provide the following information for each reciprocal translocation carrier:

1) the ratio of chromosomally balanced and unbalanced sperm and

2) the ratio of meiotic segregations responsible for the production of chromosomally unbalanced sperm (adjacent I and II, 3:1, and 4:0).

Since the hamster egg-human sperm fusion technique allows direct visualization of sperm chromosomes, results obtained should reflect the true frequencies of the different meiotic segregations (alternate, adjacent I and II, 3:1, and 4:0) occurring in the gonads.

3) the frequency of chromosomal abnormalities unrelated to the translocation. The frequency of hyperhaploidy will be doubled to give the conservative estimate of aneuploidy frequency. This figure will then be used to determine if an interchromosomal effect has occurred during meiosis in these two reciprocal translocation carriers.

4) the ratio of X- and Y-bearing sperm. In a typical XY male, the ratio of X- to Y-bearing sperm is expected to be 1:1.

III. MATERIALS AND METHODS

A. Donors

Two male reciprocal translocation carriers were unexpectedly ascertained through the IVF clinic in Birmingham, England by Dr. Maj Hulten. These men had volunteered to be sperm donors for the artificial insemination program, and in the process of screening their karyotypes, heterozygosity for a reciprocal translocation was discovered. The sperm was collected, cryopreserved, and sent to Calgary in a dry shipper containing liquid nitrogen. Studies by Chernos and Martin (1989) have shown that cryopreservation of sperm does not alter its chromosomal constitution.

B. Sample Size Considerations

One of the major issues to be studied in this research project was to determine if an interchromosomal effect is present or absent in these two reciprocal translocation carriers. The frequency of aneuploidy in normal men studied in the same laboratory is 2.4% (Martin et al., 1987). If an interchromosomal effect is present, the frequency of aneuploidy should be increased in men heterozygous for a reciprocal translocation. The numerical sample sizes required to detect a tripling or doubling of the control frequency of aneuploidy can be calculated using a one-sided, one-sample binomial test (Rosner, 1986, p.225). With a significance level of 0.05 and a power level of 0.90, the sample sizes (n) needed to

detect a tripling (3x) and a doubling (2x) in aneuploidy frequency is calculated to be:

$$n(3x) = \frac{p_o q_o \left(z_{1-a} + z_{1-\beta} \sqrt{\frac{p_1 q_1}{p_o q_o}} \right)^2}{| p_1 - p_o |^2}$$

$$n(3x) = \frac{(.024)(.976)\left[1.645 + 1.28 \sqrt{\frac{(.072)(.928)}{(.024)(.976)}}\right]^2}{| .072 - .024 |^2}$$

$$n(3x) = \underline{148}$$

$$n(2x) = \frac{p_o q_o \left(z_{1-a} + z_{1-\beta} \sqrt{\frac{p_1 q_1}{p_o q_o}} \right)^2}{| p_1 - p_o |^2}$$

$$n(2x) = \frac{(.024)(.976)\left[1.645 + 1.28 \sqrt{\frac{(.048)(.952)}{(.024)(.976)}}\right]^2}{| .048 - .024 |^2}$$

$$n(2x) = \underline{480}$$

C. Hamster Oocyte/Human Sperm Fusion Technique

Human sperm chromosomal complements were obtained by *in vitro* penetration of zona pellucida-free hamster oocytes by human sperm. This technique was pioneered by Rudak *et al.* (1978) and has been modified by Martin (1983a). It has since been further altered by Brandriff *et al.* (1985a), who discovered that TES-Tris (TEST) yolk buffer enhances sperm capability to fertilize zona pellucidae-free hamster oocytes, resulting in a higher yield of sperm karyotypes. The protocol used is described in detail below.

D. Media Preparation

Compositions of all media are detailed in Appendix A, and a complete list of reagents may be found in Appendix B.

Ackerman's cryoprotectant was used by Dr. Maj Hulten and her laboratory to cryopreserve the sperm (Behrman and Ackerman, 1969). This protective medium consists of egg yolk, glycerol, glycine, glucose, and sodium citrate in an aqueous solution. The medium was heat inactivated for 30 minutes at 56°C and its pH was adjusted to 7.2 - 7.4 with 0.1N NaOH.

Biggers-Whitten-Whittingham medium (BWW), a modified Krebs-Ringer solution was used, with some additional modifications for sperm and oocyte preparation (Biggers et al., 1971). A stock solution was made bi-monthly and the working solution was made on experiment day under sterile conditions, by adding 0.2106 g of $NaHCO_3$, 0.37 ml of DL-lactic acid, and 0.5 g of human serum albumin to 100 ml of BWW stock solution. The medium was then filter-sterilized through a cellulose acetate/nitrate membrane, pore size 0.22 um. The pH was adjusted as necessary to 7.4 with the appropriate amount of either acid or base hepes.

TES-Tris (TEST) yolk buffer was used for sperm preservation and capacitation (Brandriff et al., 1985a). Dextrose, streptomycin, penicillin, and egg yolk were added to a TES-Tris buffer and the resulting solution was centrifuged. The supernatant was decanted, its pH was

adjusted to 7.4 with solid Tris, and 1 ml aliquots were then stored at -20°C. Prior to use, the aliquot was removed from the freezer and was placed in an incubator at 37°C to thaw.

Ham's F10 medium, supplemented with 15% fetal bovine serum, penicillin, and streptomycin, was used for fertilization checks and for culture of fertilized oocytes. Under sterile conditions, 7.5 ml of heat-inactivated fetal bovine serum and 0.5 ml of penicillin-streptomycin antibiotic solution were added to 42.5 ml of Ham's F10 medium and the pH was adjusted to 7.2 with 1N HCl.

E. Human Sperm Preparation

In England, the sperm samples were obtained in sterile sample cups and were immediately placed in an incubator at 37°C to allow the samples to liquefy. The semen volume was measured and an equal amount of Ackerman's cryoprotectant was added. This mixture was drawn into 0.25 ml plastic freezing straws and the ends of the straws were plugged with polyvinylpyrollidone (PVP) powder. Freezing was accomplished by lowering the straws at a controlled rate into a cryogenics tank filled to a depth of 2 inches with liquid nitrogen. The frozen sperm samples were then placed in a dry shipper (L'Air Liquide model BT 2/R) filled with liquid nitrogen, and were sent to Calgary by air freight. Upon arrival, frozen specimens

were stored in liquid nitrogen in a large Dewar flask at a temperature of -196°C.

Twenty-four hours prior to the collection of oocytes, the cryopreserved sperm was removed from liquid nitrogen and was allowed to liquefy at room temperature. An equal volume of TEST yolk buffer was added to the cryopreservant/sperm mixture in an air-tight vial. The vial containing the TEST yolk buffer/cryopreservant/sperm mixture was placed in a container filled with water at room temperature, which was buried in crushed ice, placed in an ice chest, and stored in a refrigerator at 4°C.

Between fifteen and sixty minutes prior to experiment time, the TEST yolk buffer/cryopreservant/sperm mixture was taken out of the refrigerator and was placed in the incubator to allow it to warm up to 37°C. The warmed sperm mixture was then diluted to 10 ml with BWW medium and was centrifuged at room temperature for 6 minutes at 600xG in a clinical centrifuge to remove cryoprotectant, TEST yolk buffer, and also seminal fluids which otherwise would inhibit capacitation. The supernatant layer was discarded and the pellet was gently resuspended in 10 ml of BWW medium. Repeating the above centrifugation steps, the sperm was washed two more times. However, the supernatant from these two washes was recentrifuged to recover any additional sperm. The final pellet was resuspended in appropriate amounts of BWW medium to give a final concentration of 2 - 33 x 10^6 sperm/ml. Sperm drops

of 25 to 50 ul were placed in plastic petri dishes, covered with paraffin oil, and kept at 37°C, 5% CO_2, and 95% humidity in the incubator until oocytes were ready for co-incubation.

All preparations involving human semen were handled with gloves and were conducted in a laminar flow cabinet. All materials used for the preparation of sperm droplets were discarded in biohazard containers.

F. Hamster Oocyte Preparation

Eight female golden hamsters (Mesocricetus auratus) (Charles River Laboratory, Laval, Quebec) between the ages of 2 and 5 months were routinely used per experiment. The hamsters were kept on a light/dark schedule (8am-10pm/10pm-8am) for a minimum of one week. Three days prior to the experiment, the hamsters were injected intraperitoneally with 25 IU of pregnant mare's serum gonadotropin. An intraperitoneal injection of 25 IU of human choronic gonadotropin was administered 16 hours prior to the collection of oocytes. Each hamster yielded approximately 40 to 60 oocytes, giving a total of 300 to 500 oocytes per experiment.

The hamsters were stunned with ether and killed by cervical dislocation. The oviducts were dissected and placed in a watchglass containing BWW medium. After one wash, the oviducts were placed in another watchglass containing BWW medium and were punctured, releasing the cumulus masses into the medium. Remaining cumulus was

pulled out of the oviduct using fine forceps, and the emptied oviducts were discarded. Hyaluronidase was added to this dish to a final concentration of 0.1%. Within two to three minutes, the cumulus cells were dispersed by the hyaluronidase, and the oocytes were collected using a micropipet (made by drawing out 5 3/4- or 9-inch Pasteur pipet) attached to a mouthpiece by rubber tubing. The collected oocytes were washed three times in BWW medium, and then were transferred to BWW medium containing 0.1% trypsin. Within one minute, trypsin dissolved the zona pellucida, releasing the first polar body. The zona pellucida-free oocytes were further washed three times with BWW medium and 20 to 30 were placed into each of the prepared sperm droplets.

Petri dishes containing the oocytes in sperm droplets were incubated at 37°C, in 5% CO_2, at 95% humidity until fertilized or for three hours, whichever occurred first.

G. Fertilization Check and Oocyte Culture

After one half hour of oocyte/sperm co-incubation, fertilization checks were initiated. Approximately five to ten oocytes in an F10 drop were flattened by a coverslip supported at the corners by vaseline drops, and these flattened oocytes were examined under a phase microscope at 400x magnification. An adequate level of fertilization was considered to have occurred when one or more swollen sperm heads were observed in half of the oocytes. After fertilization, or after three hours if

fertilization did not occur, the oocytes were washed free of sperm in F10 medium and were transferred to F10 droplets (50 ul) under paraffin oil.

The oocytes were incubated in the F10 droplets for a minimum of five hours (for morning experiments) or overnight (for afternoon experiments), at which time 50 ul of F10 solution containing, respectively, 0.4 or 0.8 ug/ml of colcemid was added. The oocytes were then incubated overnight for morning experiments or a further 5 hours for afternoon experiments.

H. Fixation

A modification of Tarkowski's method was used to fix the oocytes (Tarkowski, 1966). The oocytes were transferred to a watchglass containing a hypotonic solution of 1% sodium citrate at room temperature for three to six minutes. Five to ten oocytes were placed in a small drop of hypotonic solution on a glass slide pre-cleaned with alcohol. A total of four 20 ul drops of fixative (3:1 ethanol:glacial acetic acid, freshly prepared at time of fixation) was dropped over the oocytes from a height of 1 cm. After the first drop, a gentle warm breath was applied to prevent the oocytes from rolling. The position of the oocytes was noted by circling them with a diamond pencil on the underside of the slide. Before the first drop dried, a second drop of fixative was applied. To encourage spreading of chromosomes, the edge of the drop was wiped with the

corner of a lint-free tissue. The third and fourth drops were added in the same manner as the second. After the final drop, complete evaporation of the fixative was hastened by gentle blowing.

I. Q-banding and Photography of Chromosomes

After fixation, the slides were scanned using a Zeiss phase constrast microscope and all chromosome spreads were circled using a microscope mounted-diamond etcher. Approximately two weeks after fixation, the slides were stained with 0.5% quinacrine dihydrochloride, pH 4.4 to 4.5, for 15 to 25 minutes, and were rinsed with three changes of distilled water, pH 4.4 to 4.5, for a total of 10 minutes. The slides were stored at freezer temperature until the day of photography, at which time the slides were thawed at 4°C. A coverslip with a small amount of distilled water (pH 4.4 - 4.5) was mounted on the slide and the edges were sealed with paraffin wax. All circled chromosome spreads were viewed under a Zeiss Fluorescence photomicroscope II with a 100 watt mercury bulb and were photographed with an attached Nikon M-35S camera on Kodak technical pan film 2415 (35 mm). When the photography of chromosome spreads was completed, the slides were stored in the freezer. The exposed rolls of film were developed in Kodak D-19 solution for 4 minutes, quickly rinsed in stop bath solution (1.5% acetic acid), fixed with Kodak rapid fixer solution for 2 minutes, rinsed with water, and soaked in Kodak hypoclearing solution for 30 seconds. The

developed rolls were then rinsed in water and dipped into water containing a small amount of Kodak Photo-Flo 200 solution before allowing them to dry.

J. Analysis of Sperm Karyotypes

Initially, the pictures of Q-banded chromosomes were manually printed using Kodabromide paper (F1 - F5) and Kodak Dektol solution. With the arrival of the automatic Ilford printer, the pictures were printed on Ilford Multigrade III paper. When the prints were ready for analysis, the slides were warmed to room temperature, the coverslips were popped off with a razor blade, and the slides were rinsed with methanol to remove any traces of oil and quinacrine. The slides were then stained for 5 minutes in Giesma (3 ml of Giesma stain (original azure blend type) in 50 ml of Gurr Buffer). The Q-banded chromosomes were identified on the prints and were numbered according to the International System of Human Cytogenetic Nomenclature (ISCN, 1985). Giesma-stained slides were used to assist in the analysis of difficult complements, particularly those containing structural anomalies.

IV. RESULTS

Two male reciprocal translocation carriers were fortuitously ascertained in the IVF Clinic at East Birmingham Hospital in Birmingham, England, when they volunteered to be sperm donors. The karyotypes of these two men are:

46,XY,t(11;17)(p11.2;q21.3)

46,XY,t(1;11)(p36.3;q13.1)

The translocated chromosomes are shown in Figures 3 and 4 along with their normal counterparts.

A. Reciprocal Translocation Carrier, t(11;17)

i. Family history

No family history is available for the t(11;17) heterozygote.

ii. Summary of data

A total of six experiments was conducted using the sperm of the t(11;17) carrier. An average of 34 (range 19 to 59) karyotypes per experiment was obtained for a total of 202 sperm chromosome complements. Eighteen of these chromosome spreads were non-informative regarding the segregation and were therefore excluded from the segregation analysis. Disregarding abnormalities unrelated to the translocation, 71 of 184 (38.6%) sperm karyotypes were chromosomally balanced and 113 of 184 (61.4%) chromosome spreads were chromosomally unbalanced. The frequency of chromosomal abnormalities unrelated to the translocation was 26.2% overall, 6.4% numerical, 19.3%

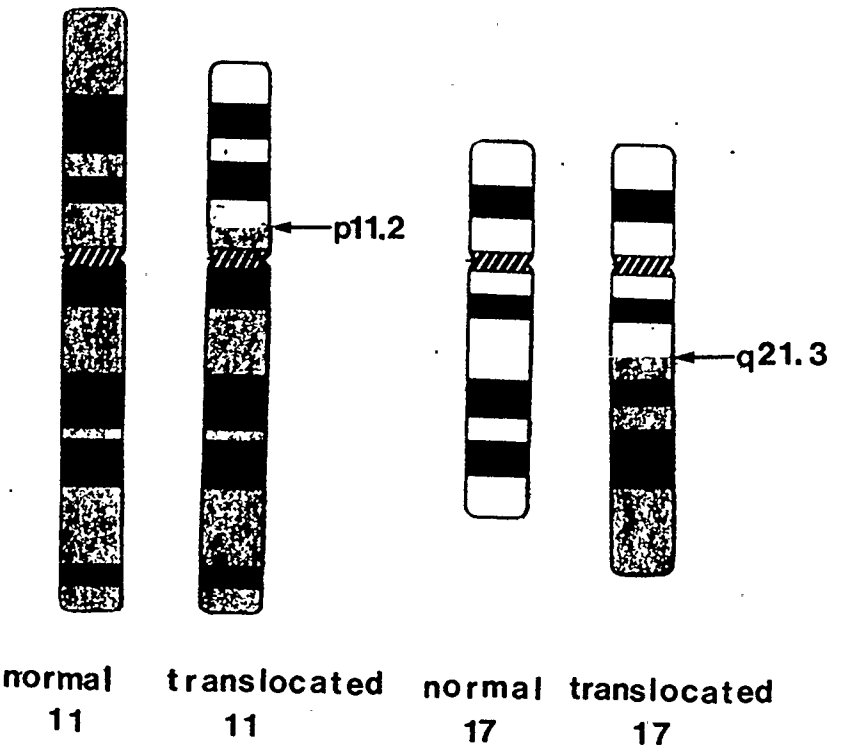

Figure 3: An idiogram showing the breakpoints in t(11;17).

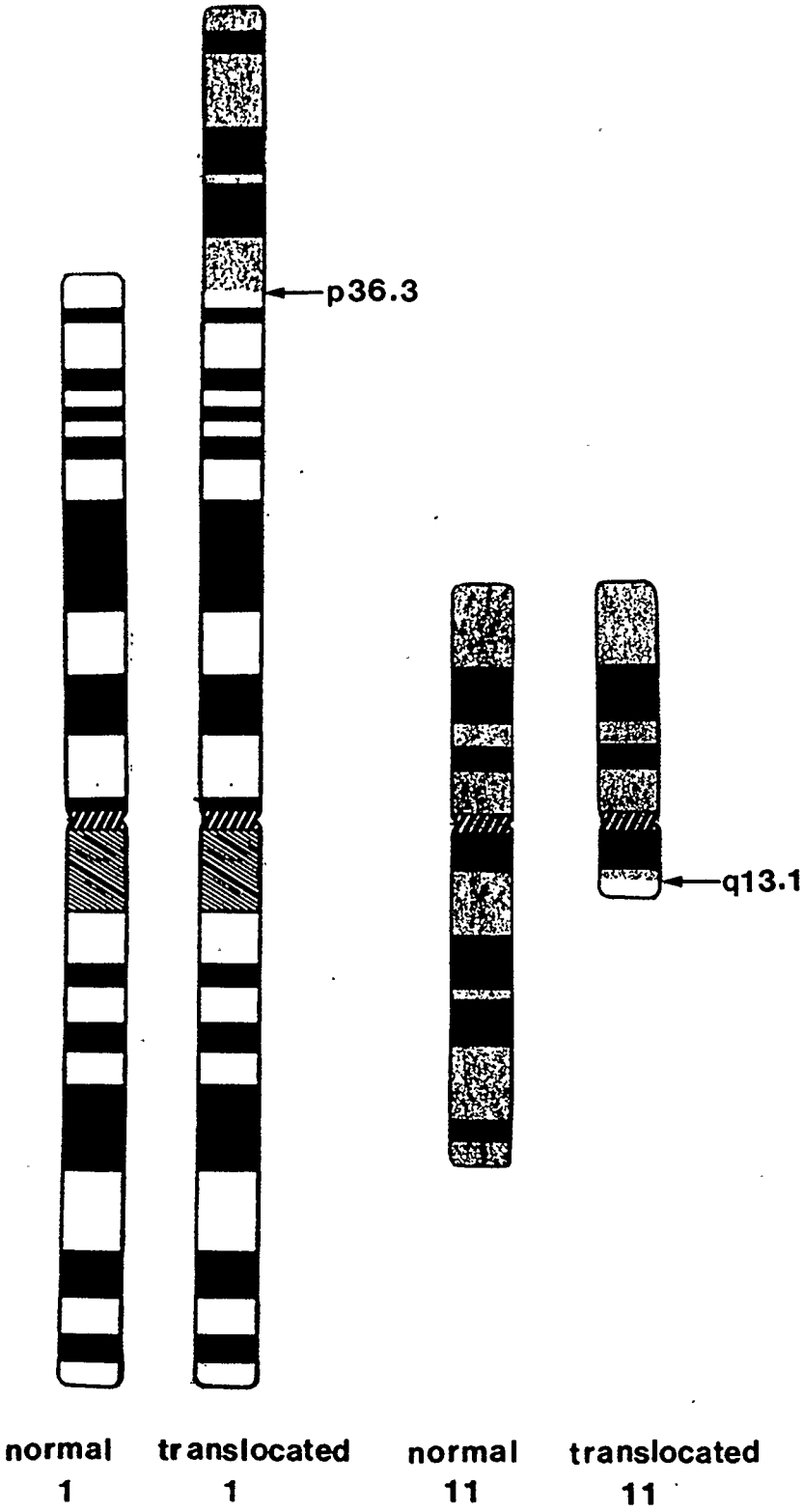

Figure 4: An idiogram showing the breakpoints in t(1;11).

structural, and 0.5% numerical and structural. The incidence of X- to Y-bearing sperm was 97:93.

iii. Abnormalities related to the translocation

Chromosome segregations are summarized in Table 3. Of the 184 karyotypes, 71 or 38.6% were chromosomally balanced, with 40 (21.7%) normal and 31 (16.8%) balanced sperm. Progeny resulting from these spermatozoa would have been phenotypically normal. An example of a balanced sperm karyotype is displayed in Figure 5. Using the Pearson's X^2 test for goodness of fit (Johnson and Bhattacharyya, 1985, p.433), the ratio of normal to balanced karyotypes is not significantly different from the expected 1:1 ratio ($X^2_{df=1}=1.1$, $p>0.1$).

The remaining 113 sperm chromosome complements were chromosomally unbalanced, and depending on the amount of chromosomal deficiency and/or duplication, these would have resulted in unrecognized or recognized spontaneous abortions, or in chromosomally abnormal progeny. Adjacent I segregation produced the majority (32.1%) of the chromosomally unbalanced sperm, followed closely by adjacent II segregation (26.6%). An example of a sperm karyotype resulting from adjacent II segregation is shown in Figure 6. Five sperm karyotypes (2.7%) were the product of 3:1 segregation and none were the product of 4:0 segregation. Theoretically, both adjacent I and II segregations should each yield two different products, in a 1:1 ratio. X^2 tests conducted on these values show that

Table 3: Segregation pattern observed in sperm of an individual heterozygous for the t(11;17) reciprocal translocation.

"Segregation" Type°	# of Spreads	%#
Alternate		
Normal		
23,X or Y	40	21.8
Balanced		
23,X or Y,-11,-17,+der(11),+der(17)	31	16.8
Total (Alternate)	71	38.6
Adjacent I		
23,X or Y,-11,+der(11)	31	16.8
23,X or Y,-17,+der(17)	28	15.3
Total (Adjacent I)	59	32.1
Adjacent II		
23,X or Y,-11,+der(17)	24	13.0
23,X or Y,-17,+der(11)	24	13.0
23,Y,-17,+11*	1	0.5
Total (Adjacent II)	49	26.6
3:1		
22,Y,-11	1	0.5
22,Y,-11,-17,+der(17)	1	0.5
24,X,+der(11)	3	1.6
Total (3:1)	5	2.7
TOTAL	184	100.0

°All unrelated abnormalities were ignored when assigning karyotypes to the appropriate segregation category. 18 karyotypes were uninformative with regard to the segregation and are not included in the above analysis.
#Percentages may not add up precisely due to rounding off.
*Crossover within the interstitial segment of chromosome 11 or nondisjunction at anaphase II.

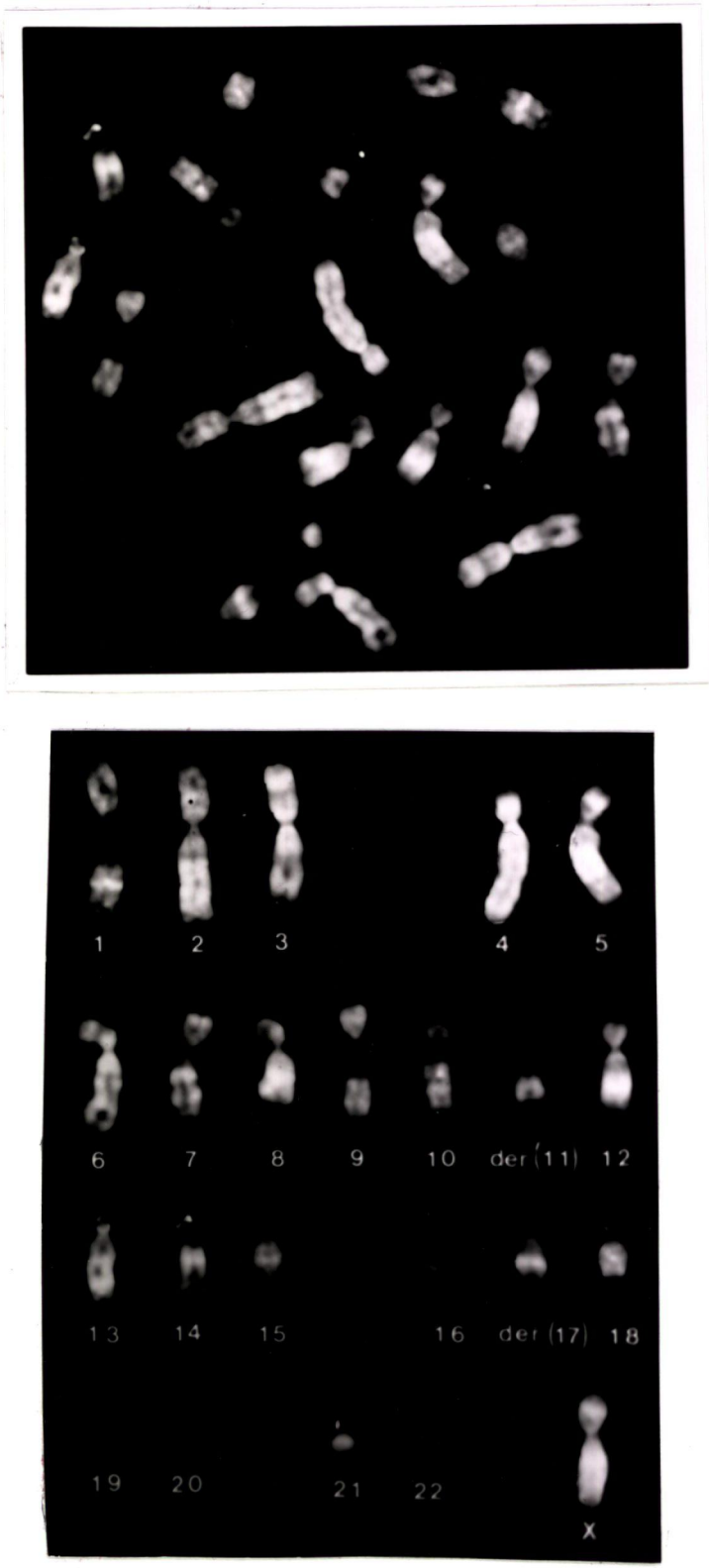

Figure 5: Q-banded karyotype of a chromosomally balanced sperm bearing both translocated chromosomes from a t(11;17) carrier. 23,X,-11,-17,+der(11),+der(17).

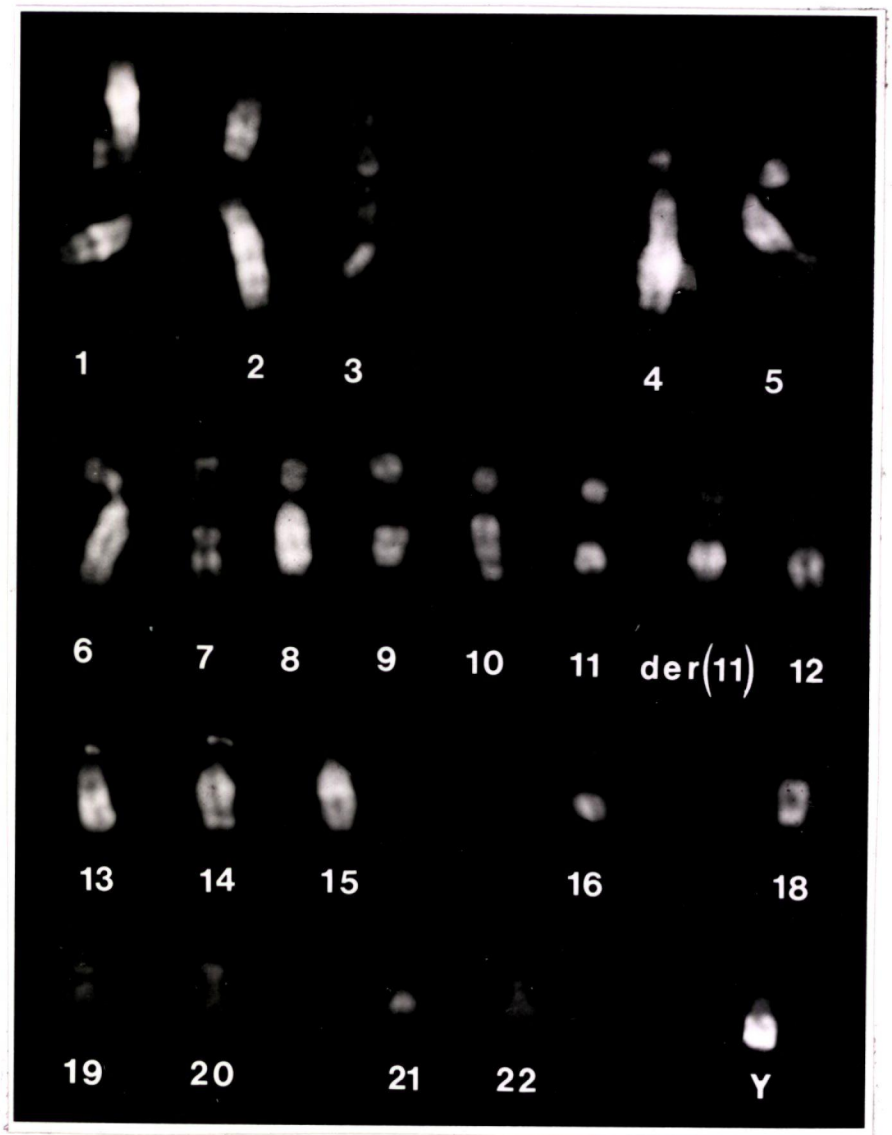

Figure 6: Q-banded karyotype of a chromosomally unbalanced sperm resulting from adjacent II segregation from a t(11;17) carrier. 23,Y,-17,+der(11).

there are no significant differences from the expected ratios.

One sperm karyotype had two normal chromosome 11's. This karyotype can be explained either by a 3:1 segregation accompanied by non-disjunction at anaphase II, or by a crossover within the interstitial region (the segment of the chromosome between the centromere and the breakpoint) followed by adjacent II segregation. While it is impossible to distinguish between the two possibilities, the latter is considered to be more likely, and thus this karyotype is included in the adjacent II category.

iv. Abnormalities unrelated to the translocation

The numerical chromosomal abnormalities unrelated to the translocation are detailed in Table 4. Of 202 sperm karyotypes, 14 or 6.9% had numerical anomalies. Of these, two (1.0%) karyotypes were double aneuploid, involving at least one missing chromosome and an extra copy of another chromosome. The remaining twelve (5.9%) were hypohaploid; no hyperhaploid sperm were observed. Hypohaploidy in this individual was observed for sex chromosomes and for representatives from Denver groups A, B, C, E, and G. Smaller chromosomes (chromosomes 16 through 22) were involved in half of the hypohaploid sperm complements. The double aneuploid karyotypes were hyperhaploid for either the sex chromosome or chromosome 1. There does not appear to be any correlation between numerical

Table 4: Sperm karyotypes with numerical abnormalities unrelated to the t(11;17) translocation.

Numerical	14/202 or 6.9%
Double Aneuploid	2/202 or 1.0%
22,-Xor-Y,-11,+der(17),-4,+1	
23,XY,-17,+der(11),-6	
Hyperhaploid	0/202 or 0.0%
Hypohaploid	11/202 or 5.4%
20,X,-11,+der(17),-4,-5,-8	
22,-Xor-Y,-11,+der(17)	
22,X,-11,+der(11),-7	
22,X,-17,+der(11),-9	
22,Y,-17,+11,-16	
22,X,-17,+der(11),-18	
22,X,-17,+der(17),-18	
22,X,-17,+der(17),-21	
22,X,-11,-17,+der(11),+der(17),-22	
22,X,-11,+der(11),-22	
22,Y,-17,+der(17),-22	
Hypohaploid and structural	1/202 or 0.5%
22,Y,-11,+der(11),-2,+ace(?part of 2)	

abnormalities and segregation type ($X^2_{df=3}=7.315$, $p>.05$; See Table 5).

Sperm karyotypes with structural abnormalities unrelated to the translocations are listed in Table 6. A total of 40 sperm chromosome complements contained structural abnormalities, representing 19.8% of the sperm karyotypes analyzed. While this percentage is high, it still falls within the range observed for normal men (Martin et al., 1987). Both simple and complex abnormalities involving chromatids and/or chromosomes were observed. Eighteen karyotypes had multiple breaks and rearrangements, rendering them uninformative with regard to the segregation. One interesting anomaly observed was the formation of an apparently balanced de novo translocation involving chromosomes 1 and 5. This spermatozoon would have had the potential to produce a different reciprocal translocation carrier, provided all essential genetic material was present and functional.

In one sperm karyotype, both numerical and structural abnormalities were observed. Thus, this chromosome spread has been counted twice in the analysis above, once in the numerical category and once in the structural category.

v. Sex ratios

Overall, the occurrence of X- and Y-bearing sperm was 97:93, which is not significantly different from the expected 1:1 ratio ($X^2_{df=1}= 0.1$, $p>0.5$). Twelve karyotypes were non-informative about the sex chromosomal

Table 5: Sperm karyotypes with numerical abnormalities unrelated to the t(11;17) translocation, divided into separate segregation categories. As expected, the frequency of aneuploidy is not dependent on the segregation.

	Observed	Expected
Alternate	1	5.4
22,X,-11,-17,+der(11),+der(17),-22		
Adjacent I	6	4.5
22,Y,-11,+der(11),-2,+ace(?part of 2)		
22,X,-11,+der(11),-7		
22,X,-11,+der(11),-22		
22,X,-17,+der(17),-18		
22,X,-17,+der(17),-21		
22,Y,-17,+der(17),-22		
Adjacent II	7	3.7
22,-Xor-Y,-11,+der(17),-4,+1		
20,X,-11,+der(17),-4,-5,-8		
22,-Xor-Y,-11,+der(17)		
23,XY,-17,+der(11),-6		
22,X,-17,+der(11),-9		
22,Y,-17,+11,-16		
22,X,-17,+der(11),-18		
3:1	0	0.4
	$X^2_{df=3}=7.315$, $p>.05$	

Table 6: Sperm karyotypes with structural abnormalities unrelated to the t(11;17) translocation.

All structural	40/202 or 19.8%
Structural and numerical	1/202 or 0.5%
22,Y,-11,+der(11),-2,+ace(?part of 2)	
Structural only	39/202 or 19.3%
22,X,csg(X)(q21or22),-2,-3,+dic(?2;?3),9q+,12q+,+2ace	
22,Y,-17,+der(17),csb(2)(?cen),del(9)(cen or p11), dic(10;13)(10pter-->10q2::13q3-->13pter),+ace	
22,Y,-11,-17,+der(11),+der(17),del(7)(q3),dic(8;14) (8pter-->8q2::14q2or3-->14pter),+ace	
23,X,+der(11),-3,-7,+dic(?3;?7),del(6)(q1),14q+,+ace	
22,Y,-11,+der(11),cte(5;13)(q1;q3)(qr,sym,complete)	
23,Y,-11,-17,+der(11),+der(17),cte(5)(q1;q2or3) (tr,complete)	
23,Y,-11,+der(17),ctg(1)(q32)	
23,X,-17,+der(17),ctg(2)(q2)(?ct del)	
23,X,csg(3)(p21),+ace	
23,X,-17,+der(11),csb(X)(q1)	
23,Y,-17,+der(11),csb(1)(q2)	
23,X,-11,+der(11),csb(2)(q31)	
23,X,-11,+der(17),csb(6)(p21.3)	
23,Y,csb(6)(p23)	
23,Y,-17,+der(17),csb(8)(q22)	
23,X,-17,+der(17),csb(15)(q2)	
23,X,-11,+der(11),csb(20)(q1)	
23,Y,-11,+der(11),+ace	
23,X,-17,+der(17),-2,-6,-8,+3mar,+3ace	
23,Y,t(1;5)(q2;q11-13)	
__,X,MB+R,[nor(17),der(17)]	
[Following karyotypes were not included in segregation data]	
__,_,MB+R	(9)
__,X,MB+R	(5)
__,Y,MB+R	(3)
__,Y,MB	

content. Within each segregation category, the X:Y ratio was not significantly different from the expected 1:1 ratio.

vi. Interchromosomal effect

For the 11;17 translocation carrier, 202 sperm chromosome complements were obtained. This sample size is sufficient to detect a tripling in the frequency of aneuploidy (see Materials and Methods for calculations). Using the hyperhaploidy observed in the double aneuploid karyotypes, a conservative estimate of the aneuploidy rate was calculated to be 2.0%. This figure is slightly less than the conservative estimate of the aneuploidy rate of 2.4% observed in the normal population (Martin et al., 1987).

B. Reciprocal Translocation Carrier, t(1;11)

i. Family history

The family pedigree is shown in Figure 7. The t(1;11) heterozygote and his brother inherited the reciprocal translocation from their father. As the proband's father and uncle have the same translocation, they too must have inherited it from one of their parents. Also, the proband has one son with the balanced form of the translocation. Hence, the reciprocal translocation has been in the family for at least four generations. Of the four couples, only two have had a history of spontaneous abortions and none have had chromosomally unbalanced children. The father of the proband has an

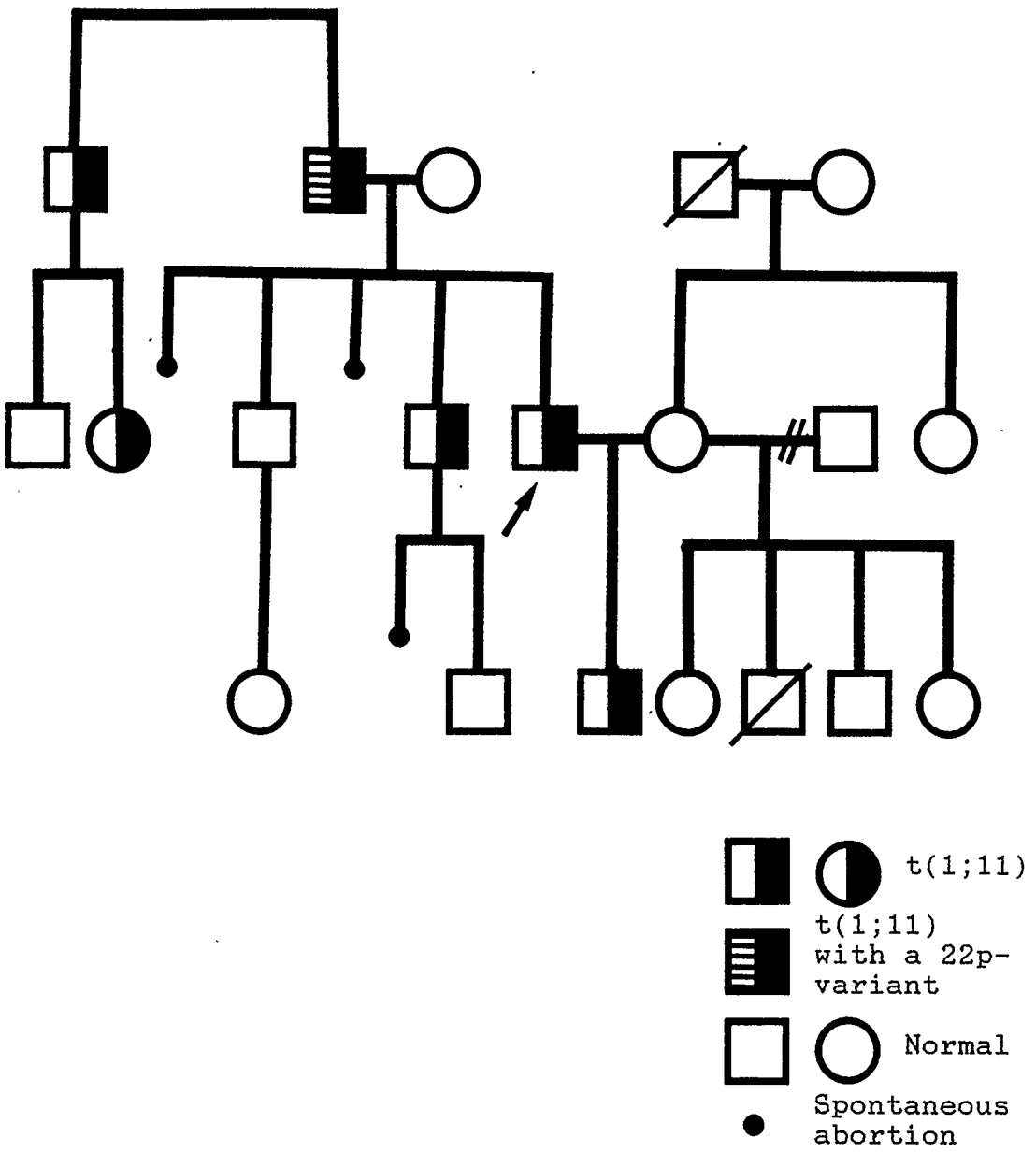

Figure 7: Family pedigree for the t(1;11) carrier.

unrelated variant, a shortening of the p arm of chromosome 22.

ii. Summary of data

Using the sperm of the translocation carrier, t(1;11), thirteen experiments were conducted. In one experiment, over-fertilization occurred and no results were obtained. From the remaining twelve experiments, 575 sperm chromosome complements were successfully analyzed. The range of sperm karyotypes obtained per experiment was 19 to 92, with an average of 48 spreads per experiment. Twenty-seven complements were not included in the segregation data, due to the presence of multiple breaks and rearrangements. Of the remaining 548 sperm karyotypes, 182 (33.2%) were chromosomally balanced and 366 (66.8%) were chromosomally unbalanced. Numerical and structural abnormalities unrelated to the translocation were present in 5.6% and 9.2%, respectively, of the sperm chromosome complements. The occurrence of X- and Y-containing sperm was 290:271.

iii. Abnormalities related to the translocation

The segregations observed are summarized in Table 7. A total of 548 haploid spreads was included in the segregation data analysis. The number of karyotypes with both normal and both translocated chromosomes was 87 (15.9%) and 95 (17.3%), respectively. These spermatozoa, the product of alternate segregation, would have resulted in phenotypically normal progeny. An example of a

Table 7: Segregation pattern observed in sperm of an individual heterozygous for the t(1;11) reciprocal translocation.

"Segregation" Type°	# of Spreads	%#
Alternate		
Normal		
23,X or Y	87	15.9
Balanced		
23,X or Y,-1,-11,+der(1),+der(11)	95	17.3
	===	====
Total (Alternate)	182	33.2
Adjacent I		
23,X or Y,-1,+der(1)	119	21.7
23,X or Y,-11,+der(11)	116	21.2
	===	====
Total (Adjacent I)	235	42.9
Adjacent II		
23,X or Y,-1,+der(11)	24	4.4
23,X or Y,-11,+der(1)	43	7.8
23,X or Y,-11,+1*	10	1.8
23,X or Y,-1,-11,+der(1),+der(1)*	10	1.8
	===	====
Total (Adjacent II)	87	15.9
3:1		
22,X or Y,-1	0	0.0
22,X or Y,-11	9	1.6
22,X or Y,-1,-11,+der(1)	5	0.9
22,X or Y,-1,-11,+der(11)	6	1.1
	20	3.6
24,X or Y,+der(1)	4	0.7
24,X or Y,+der(11)	3	0.5
24,X or Y,-1,+der(1),+der(11)	8	1.5
24,X or Y,-11,+der(1),+der(11)	4	0.7
	19	3.5
24,X or Y,-1,+der(1),+der(1)*	1	0.2
24,X or Y,-1,-11,+der(1),+der(1), +der(11)*	4	0.7
	5	0.9
	===	====
Total (3:1)	44	8.0
	===	=====
TOTAL	548	100.0

°All unrelated abnormalities were ignored when assigning karyotypes to the appropriate segregation category. 27 karyotypes were uninformative about the segregation and are not included in the above analysis.
#Percentages may not add up precisely due to rounding off.
*Crossover within the interstitial segment of chromosome 1 or nondisjunction at anaphase II.

sperm balanced for the translocation is shown in Figure 8. As expected, the frequency of normal to balanced sperm is not significantly different from a 1:1 ratio ($X^2_{df=1}=0.4$, $p>0.5$).

The remaining 66.8% of the sperm analyzed were chromosomally unbalanced, and depending upon the various deficiencies and/or duplications present, would have resulted in abnormal liveborns. Adjacent I segregation produced a significant proportion of the abnormal sperm. An example of a sperm resulting from adjacent I segregation is shown in Figure 9. The frequencies of unbalanced sperm produced as a result of adjacent II and 3:1 segregations were 15.9% and 8.0%, respectively. None of the spermatozoa was the result of 4:0 segregation.

Adjacent II segregation yielded uneven numbers of its two possible products, which was statistically significant ($X^2_{df=1}=17.5$, $p<0.01$). Twenty-four karyotypes had both chromosomes with the centromeres of chromosome 11 and 63 had both chromosomes with the centromeres of chromosome 1.

A high proportion (25 karyotypes; 4.6%) of the sperm analyzed had two normal chromosome 1's (10) or two derivative 1's (15). These karyotypes can be explained either by a non-disjunction at anaphase II or by a crossover within the interstitial region (the segment of the chromosome between the centromere and the breakpoint) followed by adjacent II or 3:1 segregation. Since non-disjunction at anaphase II is considered to be a remote

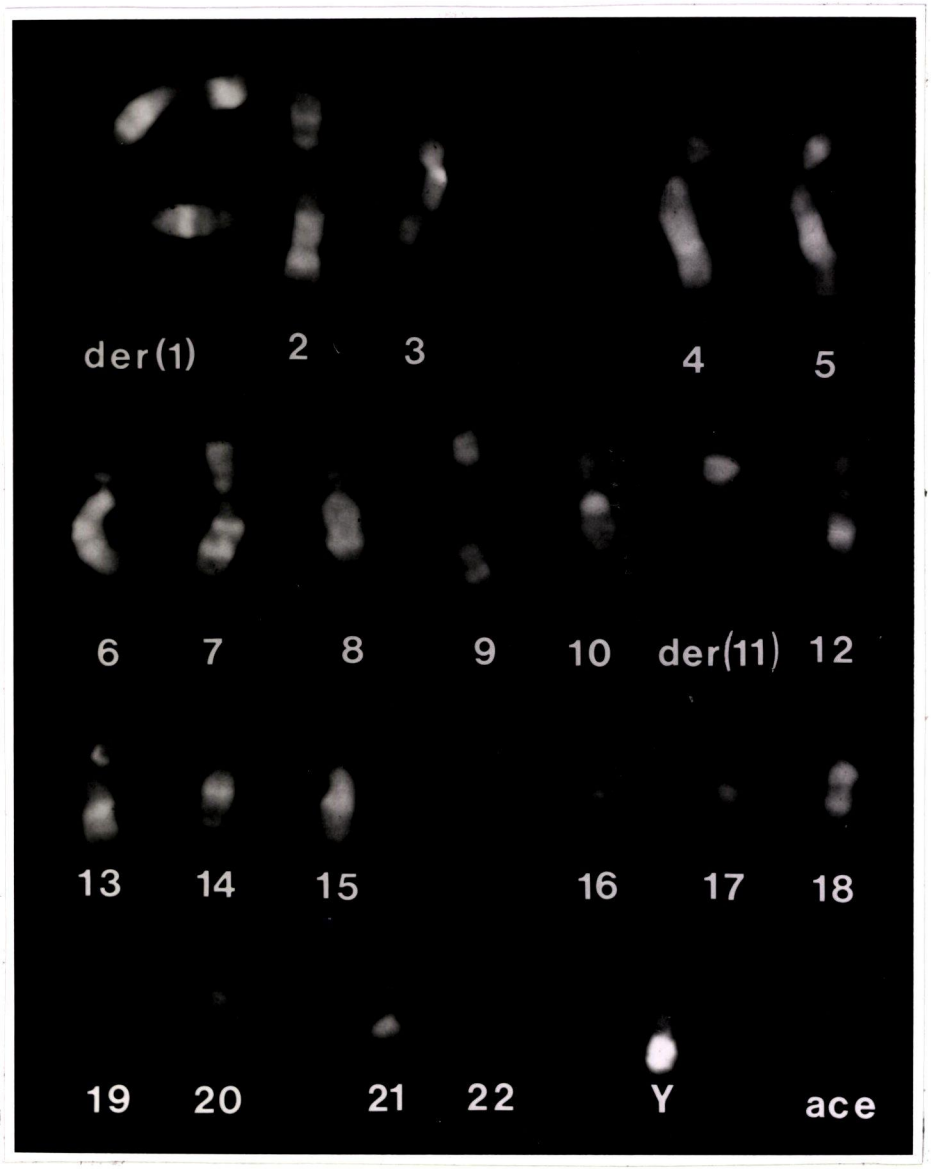

Figure 8: Q-banded karyotype of a sperm with both translocated chromosomes and an unidentified acentric fragment, from a t(1;11) carrier. 23,Y,-1,-11,+der(1), +der(11),+ace.

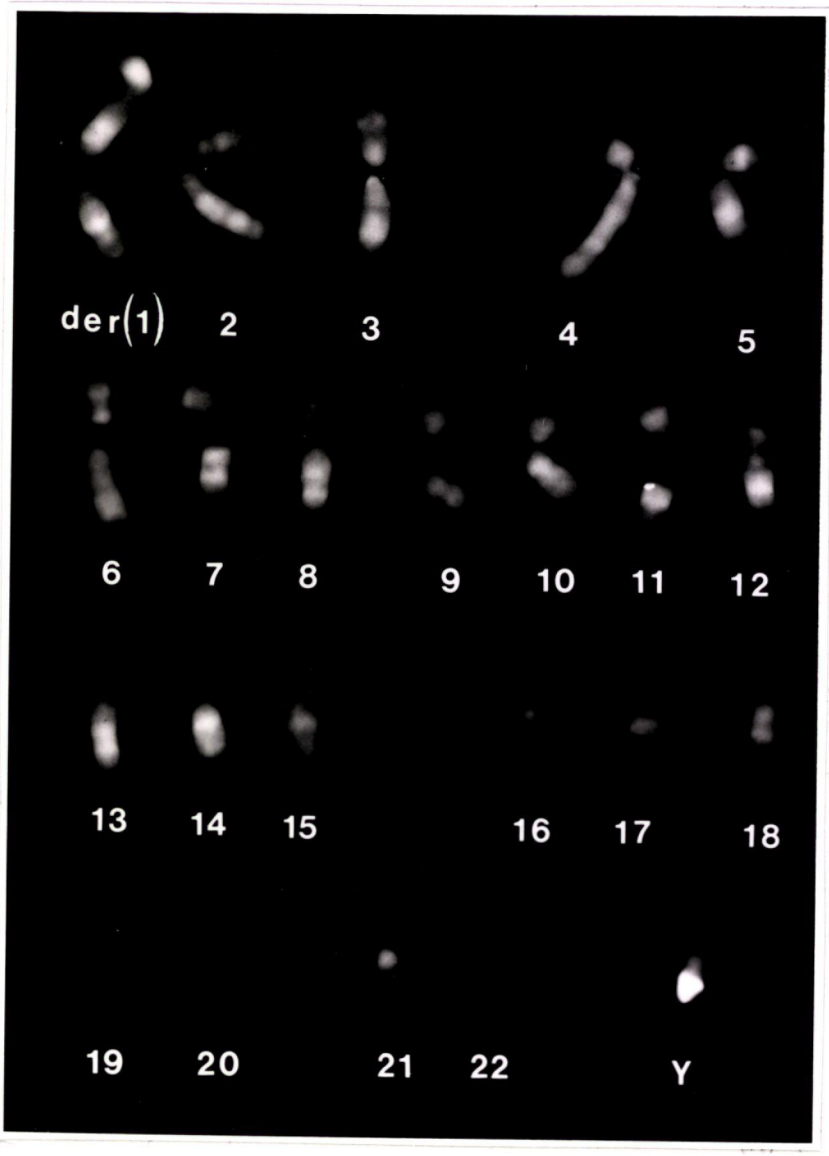

Figure 9: Q-banded karyotype of a chromosomally unbalanced sperm resulting from adjacent I segregation from a t(1;11) carrier. 23,Y,-1,+der(1).

possibility, it was assumed that these karyotypes were the result of a crossover followed either by adjacent II segregation (20 spreads) or by 3:1 segregation (5 spreads) and were included in the appropriate segregation category. An example of a sperm resulting from a crossover within the interstitial segment followed by 3:1 segregation is shown in Figure 10.

iv. Abnormalities unrelated to the translocation

A total of 85 sperm chromosome complements (14.8%) with abnormalities unrelated to the translocation was observed. Sperm karyotypes with numerical abnormalities unrelated to the translocation are listed in Table 8. Thirty-two (5.6%) had numerical abnormalities. Two (0.3%) had an extra chromosome and 30 (5.2%) were missing at least one chromosome. Hyperhaploidy was observed for chromosomes Y and 21. Ignoring the translocated chromosomes, if these hyperhaploid sperm had fertilized an egg, an XYY male and a trisomy 21 conceptus would have resulted (the 24,YY sperm karyotype is shown in Figure 11). Hypohaploidy was observed for sex chromosomes and for representatives from all Denver groups. Again, half of the hypohaploid sperm were missing the smaller chromosomes (chromosomes 16 to 22). Loss or gain of a chromosome occurred randomly in all segregation categories ($X^2_{df=3}=0.33$, $p>.95$) (See Table 9).

Sperm chromosome complements with structural abnormalities unrelated to the translocation are detailed

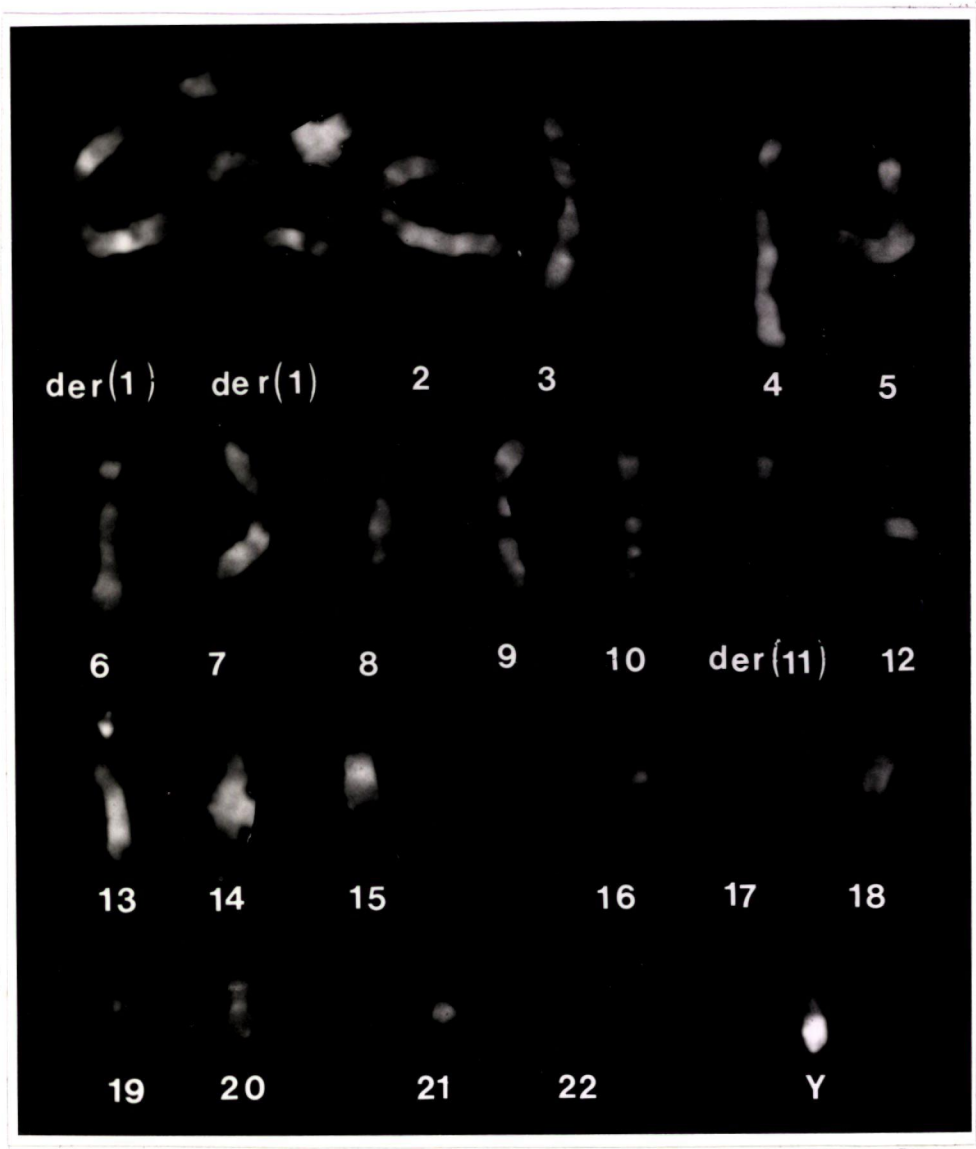

Figure 10: Q-banded karyotype of a chromosomally unbalanced sperm resulting from a crossover in the interstitial segment followed by 3:1 segregation, from a t(1;11) carrier. 23,Y,-1,-11,+der(1),+der(1),+der(11).

Table 8: Sperm karyotypes with numerical abnormalities unrelated to the t(1;11) translocation.

Total Numerical	32/575 or 5.6%
Double Aneuploid	0/575 or 0.0%
Hyperhaploid	2/575 or 0.3%
24,YY	
24,X,-11,+der(1),+21	
Hypohaploid	30/575 or 5.2%
20,Y,-11,+der(11),-18,-20,-22	
20,Y,-11,-3,-6	
21,X,-1,+der(1),-8,-22	
21,-Xor-Y,-1,-11,+der(11)	
22,-Xor-Y,-1,+der(1)	
22,-Xor-Y,-11,+der(11)	
22,X,-11,+der(11),-3	
22,Y,-11,+der(1),-5	
22,Y,-11,+der(1),-6	
22,Y,-9or-10	
22,X,-11,+der(11),-10	
22,Y,-1,+der(1),-12	
22,X,-1,+der(1),-D	
22,Y,-13	
22,X,-11,+der(11),-14	
22,Y,-1,-11,+der(1),+der(11),-14or-15	
22,Y,-15	
22,X,-16	
22,Y,-1,-11,+der(1),+der(11),-16	
22,X,-1,+der(1),-17	
22,Y,-11,+der(11),-18	
22,Y,-11,+der(1),-18	
22,X,-19	
22,X,-11,+der(11),-19	
22,X,-11,+der(11),-19	
22,X,-1,-11,+der(1),+der(11),-19	
22,Y,-19	
22,X,-20	
22,X,-1,+der(1),-21	
22,X,-11,+der(11),-22	

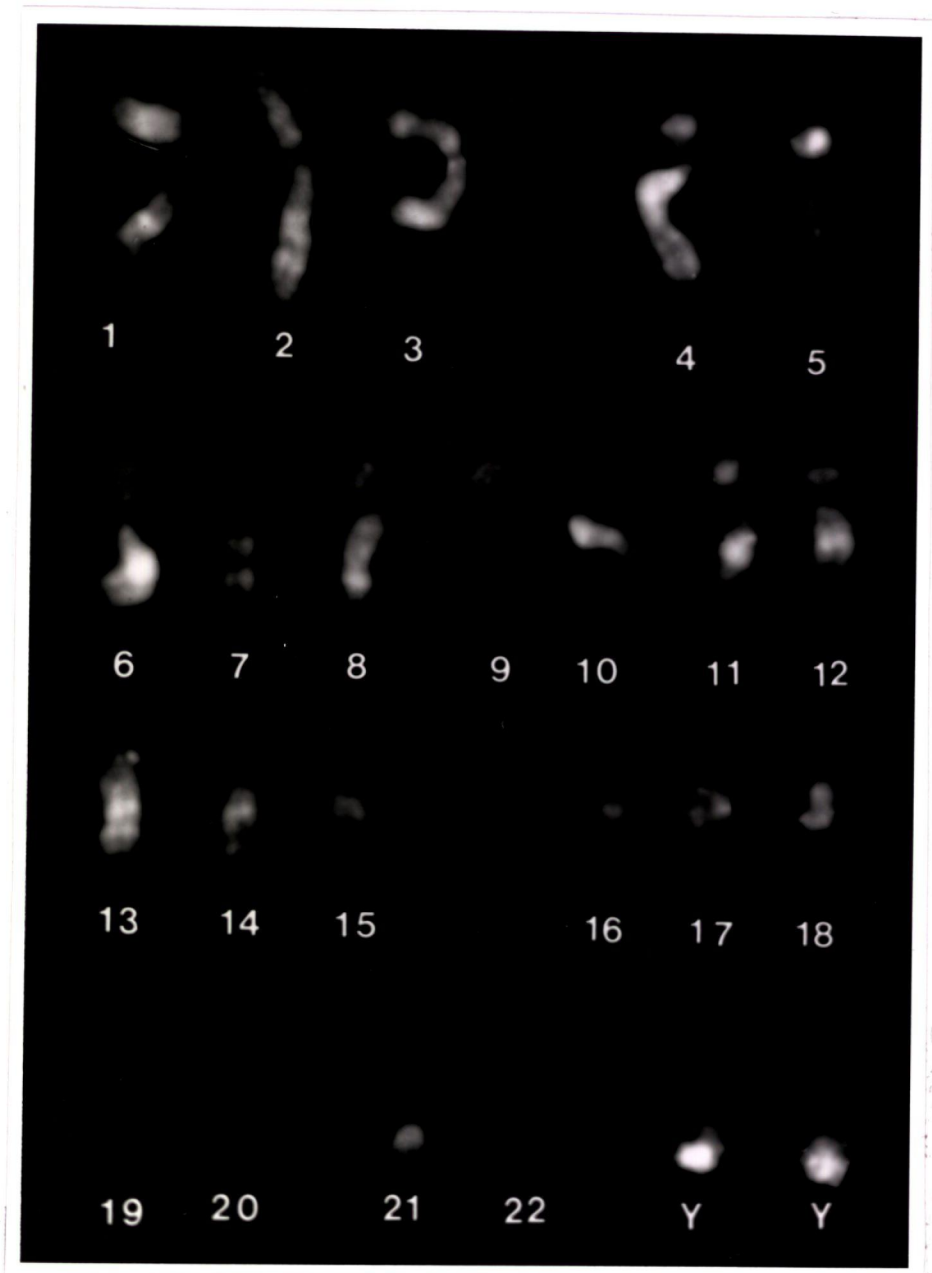

Figure 11: Q-banded karyotype of a sperm with an extra Y chromosome from a t(1;11) carrier. 24,YY.

Table 9: Sperm karyotypes with numerical abnormalities unrelated to the t(1;11) translocation, divided into separate segregation categories. Incidence of numerical abnormalities occurred randomly in all segregation groups.

	Observed	Expected
Alternate	11	10.6
24,YY		
22,Y,-9or-10		
22,Y,-13		
22,Y,-15		
22,X,-16		
22,X,-19		
22,Y,-19		
22,X,-20		
22,Y,-1,-11,+der(1),+der(11),-14or-15		
22,Y,-1,-11,+der(1),+der(11),-16		
22,X,-1,-11,+der(1),+der(11),-19		
Adjacent I	14	13.7
21,X,-1,+der(1),-8,-22		
22,-Xor-Y,-1,+der(1)		
22,Y,-1,+der(1),-12		
22,X,-1,+der(1),-D		
22,X,-1,+der(1),-17		
22,X,-1,+der(1),-21		
20,Y,-11,+der(11),-18,-20,-22		
22,-Xor-Y,-11,+der(11)		
22,X,-11,+der(11),-3		
22,X,-11,+der(11),-14		
22,Y,-11,+der(11),-18		
22,X,-11,+der(11),-19		
22,X,-11,+der(11),-19		
22,X,-11,+der(11),-22		
Adjacent II	4	5.1
24,X,-11,+der(1),+21		
22,Y,-11,+der(1),-5		
22,Y,-11,+der(1),-6		
22,Y,-11,+der(1),-18		
3:1	3	2.6
20,Y,-11,-3,-6		
21,-Xor-Y,-1,-11,+der(11)		
22,X,-11,+der(11),-10		
	$X^2_{df=3}=0.33$, $p>.95$	

in Table 10. Structural anomalies were present in 53 sperm chromosome spreads. This figure (9.2%) is well within the range of the frequencies of structural abnormalities observed in normal males (Martin et al., 1987). Multiple breaks and rearrangement were present in 28 of the 53 sperm karyotypes with structural anomalies; only one had sufficient information to be included in the segregation data. Other anomalies observed involved simple and complex aberrations of chromatids and/or chromosomes. An example of a sperm karyotype with an unidentified acentric fragment is shown in Figure 8.

v. Sex ratios

No significant deviation from the expected 1:1 ratio was observed in the sex chromosome frequency (290 X:271 Y; $X^2_{df=1}=0.6$, $p>0.1$). Fourteen karyotypes were not included in the sex ratio due to lack of information about the sex chromosome content. Within each segregation category, the frequency of X- and Y-bearing sperm did not significantly differ from the expected 1:1 ratio.

vi. Interchromosomal effect

A sample size of 575 sperm karyotypes meets the criteria necessary for the detection of a doubling in the aneuploidy rate (see Materials and Methods for calculations). The conservative estimate of the aneuploidy rate, (calculated by doubling the hyperhaploidy rate), is 0.7% for the t(1;11) heterozygote. This frequency is lower than the conservative estimate of the

Table 10: Sperm karyotypes with structural abnormalities unrelated to the t(1;11) translocation.

All Structural	53/575 or 9.2%
22,Y,-1,+der(1),cte(der(1);3)(1p36or11q13;3p21)(qr;asy;incomplete)	
23,Y,-1,+der(1),+der(11),del(11)(?cen or p11),cte(9;20)(q3;q1)(tr,complete)	
23,Y,-1,-11,+der(1),+der(11),ctg(3)(p1)	
23,Y,-11,+der(11),csg(7)(q32)	
23,Y,-1,-11,+der(1),+der(1),csg(9)(q12or13),csg(19)(q13.1)	
23,Y,csg(11)(q23)	
23,X,-1,-11,+der(1),+der(11),csg(19)(q13)	
23,X,-1,+der(1),csb(X)(q21)	
22,X,-11,csb(4)(p13or14)	
23,XorY,-11,+der(11),csb(4)(cen)	
23,Y,-11,+der(1),csb(5)(q12)	
23,Y,-1,+der(1),csb(5)(q2)	
23,X,-11,+der(11),(2 or 3 csb involving 6 and/or 7)	
23,Y,csb(7)(cen)	
23,X,csb(7)(q11),+ace	
23,X,-1,+der(1),csb(10)(q21)	
23,Y,csb(11)(q23)	
23,X,csb(13)(cen)	
23,X,-1,+der(1),csb(15)(q21or22)	
23,X,-11,+der(1),csb(17)(cen)	
23,Y,-1,-11,+der(1),+der(11),+ace	
23,Y,-1,-11,+der(1),+der(11),+ace(?csb(B)(q))	
23,Y,-1,+der(1),del(der(1))(1q24or25)	
23,X,-1,-11,+der(1),+der(11),del(B)(p1)	
23,Y,-1,+der(1),del(15)(q1)	
__,X,MB+R,[nor(1),der(11)]	
[Following karyotypes were not included in segregation data]	
23,X,-5,-11,-13,+3mar,+2ace,del(12)(p)	
22,X,MB+R,(involving 1,7,20,?B,?D)	
__,_,MB+R	(11)
__,X,MB+R	(5)
__,Y,MB+R	(7)
__,X,MB	(2)

aneuploidy frequency of 2.4% observed in the control population (Martin et al., 1987).

V. DISCUSSION

A. Chromosome Segregations

i. 2:2 segregations

Alternate segregation, the only segregation that produces chromosomally balanced gametes, was responsible for 38.6% and 33.2% of the sperm chromosome complements analyzed for t(11;17) and t(1;11), respectively. As theoretically expected, the ratio of normal to balanced karyotypes was not significantly different from a 1:1 ratio for either reciprocal translocation carrier. Of the 23 reciprocal translocations studied, only one, t(5;11), had a significant deviation in the normal to balanced ratio from the expected 1:1 ratio (See Table 11). This translocation was carried by an individual who is a double reciprocal translocation heterozygote (Burns et al., 1986). This significant deviation may be explained by a small sample size of 23 sperm karyotypes or by interference between the two translocations (Martin et al., 1990b). As Pellestor et al. (1989) conclude, the observation of equal numbers of balanced and normal karyotypes demonstrates that the human sperm/hamster oocyte fusion technique is a valid system for determining the frequencies of the various segregations.

Chromosomally unbalanced children of reciprocal translocation heterozygotes are frequently the result of adjacent I segregation, as this segregation often leads to the least imbalanced gametes (Jalbert et al., 1980).

Table 11: Frequency of chromosomally balanced sperm from 22 reciprocal translocation heterozygotes.

Translocation Observed	# of Spreads	Alternate segregations (%) Normal	Balanced	Total
(11;22)[1]	13	7.6	15.4	23.1
(6;14)[2]	19	42.1	26.3	68.4
(7;14)[3]	19	31.6	21.1	52.6
(5;11)[4a]	23	60.9	8.7	69.6*
(7;14)[4a]	23	17.4	13.0	30.4
(5;18)[2]	32	43.8	34.4	78.1
(5;13)[5]	57	42.1	35.1	77.2
(4;17)[5]	60	25.0	31.7	56.7
(2;5)[6b]	75	9.3	10.7	20.0
(9;18)5	82	20.7	13.5	34.1
(6;7)[5]	84	21.4	29.8	51.2
(1;2)[7]	105	23.8	17.1	41.0
(12;20)[8]	113	22.1	24.7	46.8
(15;22)[9]	147	21.1	12.9	34.0
(4;6)[3]	158	24.1	21.5	45.6
(9;10)[10]	168	20.8	19.6	40.5
(16;19)[9]	172	18.6	20.9	39.5
(3;16)[11]	201	19.9	17.4	37.3
(11;17)[12]	202	21.7	16.8	38.6
(2;9)[3]	208	25.0	18.3	43.3
(8;15)[11]	226	19.0	18.1	37.2
(3;11)[9]	262	24.4	23.3	47.7
(1;11)[12]	575	15.9	17.3	33.2

[a]Both translocations are present in one male.
[b]Some alternate and adjacent 1 segregations could not be distinguished. The percentage of alternate segregation represents the minimum.
*Normal to balanced ratio is significantly different from the expected 1:1 ratio.
[1]Martin, 1984
[2]Balkan and Martin, 1983a
[3]Martin *et al.*, 1990b
[4]Burns *et al.*, 1986
[5]Pellestor *et al.*, 1989
[6]Templado *et al.*, 1988
[7]Templado *et al.*, 1990
[8]Martin *et al.*, 1990a
[9]Martin, personal communication
[10]Martin, 1988a
[11]Brandriff *et al.*, 1986a
[12]Spriggs, this report

Similarly, adjacent I segregation has been observed in every reciprocal translocation studied, and has been responsible for a majority of the unbalanced sperm in all but one translocation (See Table 12). In this one case, the t(16;19) heterozygote, there was slightly more adjacent II segregation (31.4%) than adjacent I segregation (27.9%) (Martin, personal communication). On average, adjacent I segregation is seen in 37.9% of the sperm, with a range of 15.6% to 63.4%. The frequency of adjacent I segregation in the t(11;17) and t(1;11) carriers was 32.1% and 42.9%, respectively. Both figures are within the overall range, and inclusion of these figures did not change the mean.

Adjacent II is the next most prevalent segregation for producing chromosomally unbalanced gametes. Prior to the study of t(11;17) and t(1;11), the mean frequency of adjacent II segregation was 9.4%, with a range of 0% to 31.4%. For t(11;17) and t(1;11), the frequencies of adjacent II segregation were 26.6% and 15.9%, respectively. Both of these values are greater than the mean, but within the range, and when incorporated, the mean for adjacent II segregation rises to 10.4% (See Table 12). Although adjacent II, like adjacent I, should yield two different products in equal numbers, analysis of sperm chromosome complements from the t(1;11) carrier revealed more karyotypes with both centromeres of chromosomes 1

Table 12: Frequency of sperm carrying various unbalanced meiotic segregations for 23 different reciprocal translocations.

Translocation Observed	# Spreads	Segregations observed (%) AdjI	AdjII	3:1	4:0	Total Unbalanced
(11;22)[1]	13	38.5	23.1	15.4	0.0	76.9
(6;14)[2]	19	31.6	0.0	0.0	0.0	31.6
(7;14)[3]	19	31.6	15.8	0.0	0.0	47.4
(5;11)[4b]	23	26.1	0.0	4.3	0.0	30.4
(7;14)[4b]	23	47.8	0.0	17.4	4.3	69.5
(5;18)[2]	32	15.6	0.0	3.1	0.0	18.8
(5;13)[5]	57	21.1	1.7	0.0	0.0	22.8
(4;17)[5]	60	35.0	6.7	1.6	0.0	43.3
(2;5)[6a]	75	31.3	5.3	21.3	0.0	57.9
(9;18)[5]	82	63.4	0.0	2.4	0.0	65.8
(6;7)[5]	84	48.8	0.0	0.0	0.0	48.8
(1;2)[7]	105	41.9	5.7	11.4	0.0	59.0
(12;20)[8]	113	41.6	9.7	1.8	0.0	53.1
(15;22)[9]	147	39.5	22.4	4.1	0.0	66.0
(4;6)[3]	158	51.9	1.9	0.6	0.0	54.4
(9;10)[10]	168	48.2	5.4	5.9	0.0	59.5
(16;19)[9]	172	27.9	31.4	1.2	0.0	60.5
(3;16)[11]	201	41.3	16.4	5.0	0.0	62.7
(11;17)[12]	202	32.1	26.6	2.7	0.0	61.4
(2;9)[3]	208	28.4	24.1	4.3	0.0	56.7
(8;15)[11]	226	38.1	21.2	3.5	0.0	62.8
(3;11)[9]	262	45.8	5.7	0.8	0.0	52.3
(1;11)[12]	575	42.9	15.9	8.0	0.0	66.8
Mean	131	37.9	10.4	5.0	0.2	53.4

[a]Some alternate and adjacent 1 segregations could not be distinguished, so half of the unknown segregation percentage was added to both adjacent I segregation and total unbalanced values.
[b]Both translocations are present in one male.
[1]Martin, 1984
[2]Balkan and Martin, 1983a
[3]Martin et al., 1990b
[4]Burns et al., 1986
[5]Pellestor et al., 1989
[6]Templado et al., 1988
[7]Templado et al., 1990
[8]Martin et al., 1990a
[9]Martin, personal communication
[10]Martin, 1988a
[11]Brandriff et al., 1986a
[12]Spriggs, this report

than karyotypes with both centromeres of chromosome 11 ($X^2_{df=1}=17.5, p<.01$).

ii. 3:1 and 4:0 segregations

Like adjacent II segregation, 3:1 segregation is not always observed. On average, 5.0% of sperm analyzed were the result of 3:1 disjunction, with a range of 0% to 21.3% (See Table 12). The frequency of 3:1 segregation was 2.7% and 8.0% for t(11;17) and t(1;11), respectively. Both figures fall within the range observed in other translocation carriers and incorporation of these values did not change the mean frequency of 3:1 segregation. All four types of gametes were observed, which would lead to conceptuses with tertiary trisomy and monosomy, as well as interchange trisomy and monosomy. For the t(1;11) carrier, a large number of gametes analyzed (n=44) were the result of 3:1 segregation. Theoretically, the quadrivalent may divide in four ways to produced 3:1 gametes. In the t(1;11) carrier, all four divisions were observed and the frequencies of these four divisions were not significantly different from the expected random ratio of 1:1:1:1.

Studies of sperm from the t(11;17) and t(1;11) heterozygotes did not reveal any 4:0 segregations. To date, only one gamete with a 4:0 segregation has been observed and this sperm was missing all four chromosomes of the quadrivalent (Burns et al., 1986). Until one sperm with all four chromosomes of the quadrivalent is observed,

there will be doubt as to whether the 4:0 segregation observed was merely an artifact of scattered chromosomes. Nevertheless, 4:0 segregation, if it does occur, is extremely rare.

iii. Crossing-over in the interstitial segment

Karyotypes with two identical copies of a chromosome involved in the translocation were observed in the sperm of both t(1;11) and t(11;17) carriers. While it is possible that these sperm are the result of nondisjunction at anaphase II, it is considered more likely that these are due to crossovers in the interstitial segment, the region between the centromere and the breakpoint, followed by either adjacent II or 3:1 segregation (See Figure 12). Because nondisjunction is a relatively rare event and crossovers are common, the observance of 25 (4.6%) karyotypes containing two normal chromosome 1's or two translocated chromosome 1's is better explained by crossovers in the interstitial segement. Such crossovers produce two identical chromosomes that are each carrying one normal chromatid and one translocated chromatid. If both of these chromosomes segregate together as in adjacent II or 3:1 disjunction, there is a possibility that identical chromatids will then segregate together during meiosis II. If such crossovers are accompanied by alternate segregation, half of the gametes will be unchanged, while the other half will appear to be adjacent I segregation. Likewise, adjacent I segregations with

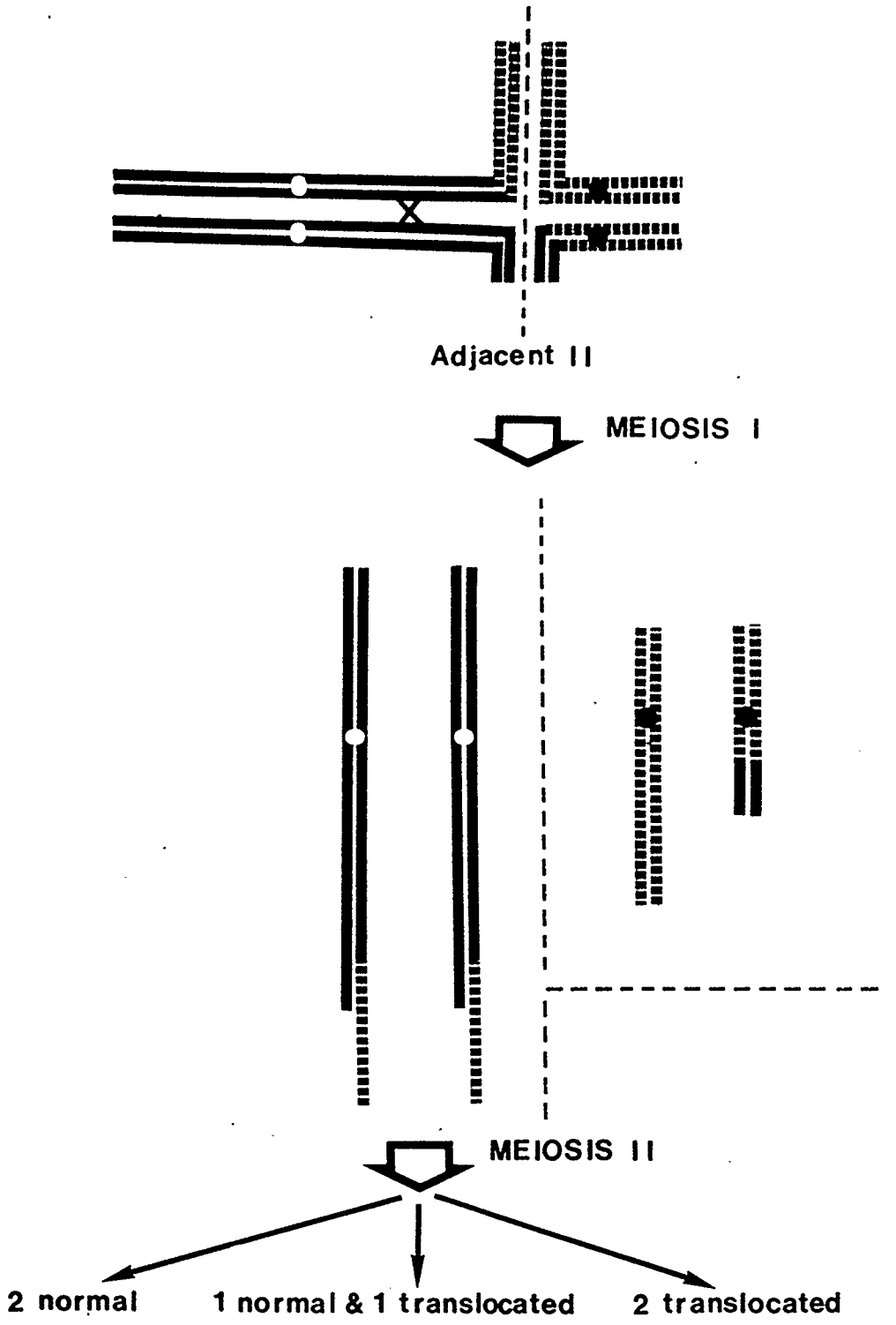

Figure 12: Crossing-over in the interstitial segment.

such crossovers will appear to be alternate segregation gametes half of the time. However, crossovers in the interstitial segment should occur randomly and thus, the number of converted gametes should be equally represented in the alternate and adjacent I categories. The presence of two identical copies of a chromosome involved in the translocation has been noted in the sperm chromosome complements for some of the other reciprocal translocation carriers studied (Martin, 1984, Brandriff etal., 1986, Martin, 1988a, Templado et al., 1988, Martin et al., 1990a, Martin et al., 1990b, Martin, personal communication). The frequency of karyotypes containing two identical copies of a chromosome involved in the translocation appears to be dependent on two factors. First, as the length of the interstitial segment increases, so does the likelihood that a crossover will occur within that region. Secondly, distinctive gametes are produced only when these crossovers are followed by adjacent II or 3:1 segregations. Hence, a translocation producing a low frequency of adjacent II and/or 3:1 segregation(s) would have few or no crossovers observed, while a translocation producing a high frequency of adjacent II and/or 3:1 segregation(s) would be more likely to have several crossovers observed.

To determine if a relationship exists between the frequency of crossovers and the length of the interstitial segment, and between the frequency of crossovers and the

frequency of adjacent II and 3:1 segregations, the correlation coefficients (r; Johnson and Bhattacharyya, 1985, p.64) were calculated. To prevent bias, reciprocal translocation carriers with an insufficient sample size (less than 75 sperm karyotypes) were excluded from both computations, and reciprocal translocations producing less than 5% adjacent II and 3:1 segregations were excluded from the first calculation. The correlation between the frequency of crossovers observed and the length of the interstitial segment was only slightly positive (r=+0.26) and was not statistically significant (Rohlf and Sokal, 1981, p.168) (See Figure 13). The correlation coefficient (r) for the relationship between the frequency of crossovers observed and the percentage of adjacent II and 3:1 segregations was calculated to be +0.29, which is not statistically significant (Rohlf and Sokal, 1981, p.168) (See Figure 14).

Unlike sperm chromosome studies, analysis of meiotic chromosomes can reveal the exact frequency and distribution of crossovers by examining chiasma formation at diakinesis (Hulten, 1974). Such studies have been conducted on chromosomally normal males (Hulten, 1974, Laurie and Hulten, 1985a) and on three fertile males carrying balanced reciprocal translocations, t(1;22) (Palmer and Hulten, 1983), t(9;10) (Laurie et al., 1984; Laurie et al., 1985), and t(Y;10) (Laurie et al., 1984). It has been shown that bivalents in normal men have

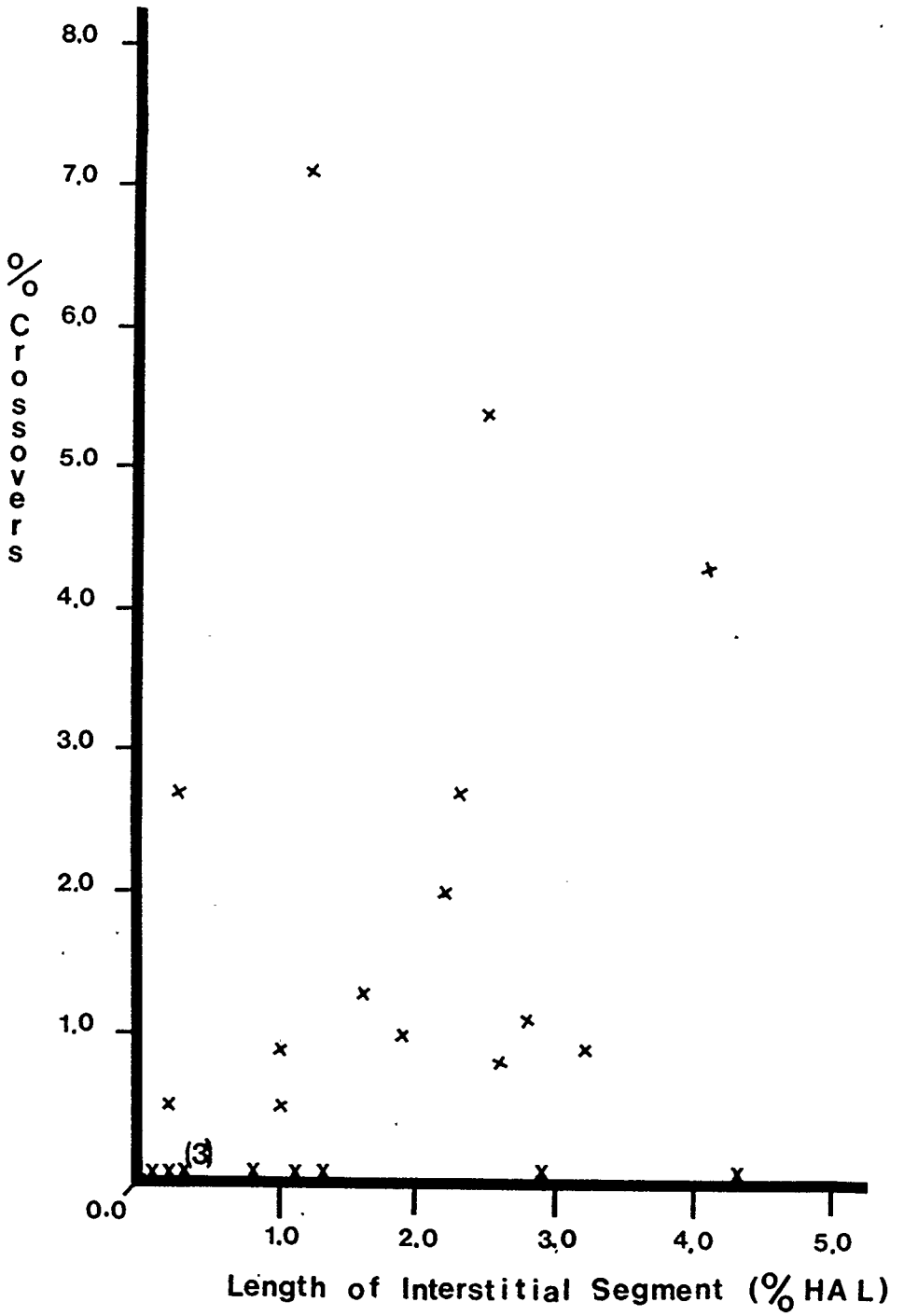

Figure 13: The relationship between the observed frequency of crossovers and the length of the interstitial segment. Bracketed value denotes the number of data values at this point. Data from the following translocations were included: t(2;5) (Templado et al., 1988), t(1;2) (Templado et al., 1990), t(12;20) (Martin et al., 1990a), t(15;22), t(16;19), and t(3;11) (Martin, personal communication), t(2;9) (Martin et al., 1990b), t(9;10) (Martin, 1988a), t(3;16) and t(8;15) (Brandriff et al., 1986a), t(11;17) and t(1;11) (Spriggs, this report).

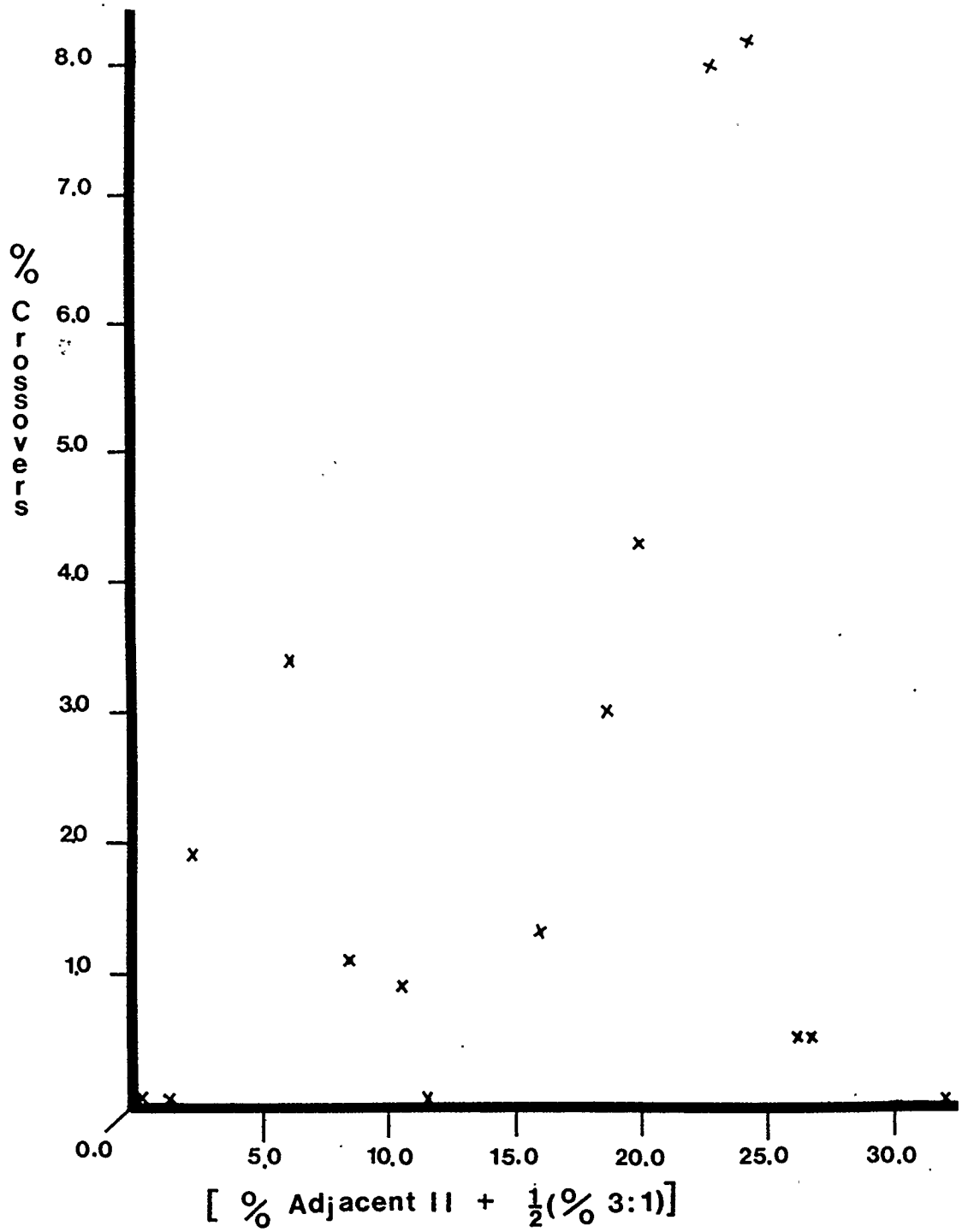

Figure 14: The relationship between the frequency of crossovers and the frequency of adjacent II and 3:1 segregations. Only half of the 3:1 segregation frequency was used as gametes with one chromosome will not show any signs of crossing-over. Data from Figure 13 and from the following translocations were included: t(9;18) and t(6;7) (Pellestor et al., 1989), t(4;6) (Martin et al., 1990b)

characteristic patterns of chiasma frequency and chiasma distribution. (Laurie and Hulten, 1985a, Laurie and Hulten, 1985b). Comparison of chiasma frequencies between males heterozygous for reciprocal translocations and normal men showed that such rearrangements increase the chiasma frequency for the chromosomes involved in the quadrivalent (Palmer and Hulten, 1983, Laurie et al., 1984, Laurie et al., 1985). However, this change appeared to be localized in only one of the two interstitial segments and the rest of the quadrivalent showed no changes in either the frequency or distribution of chiasmata.

Perhaps the poor correlation between the frequency of crossovers observed in sperm chromosome complements and the length of the interstitial segment is the result of the localized change of the chiasma frequency in the quadrivalent. With this increased chiasma frequency in only one interstitial segment, the number of crossovers seen would not only be dependent upon the length of the interstitial segment, but also dependent upon which interstitial segment was affected. Also, chiasmata appear to occur in rather fixed positions along individual bivalents in normal males (Chandley and Mitchell, 1988). Provided that chiasma frequency and distribution was not affected in the second interstitial segment, another factor affecting the number of crossovers observed would be whether this interstitial segment contained a common

chiasma site. If so, crossovers would be frequently observed, and if not, they would be rarely observed. Hence, the frequency of crossovers is dependent on factors other than the length of the interstitial segment.

At least two cases of human liveborns have been reported in which a crossover occurred in the interstitial segment of a translocated chromosome, resulting in a unique karyotype. Priest et al. (1985) reported a male reciprocal translocation carrier, t(9;10), in which the translocated chromosome 9 also exhibited a pericentric inversion. Two of his children carried the balanced form of the translocation, but the translocated chromosome 9 was not inverted. The investigators then concluded that a crossover had occurred in the interstitial segment, followed by adjacent I segregation. The second case involved a commonly-observed t(11;22) translocation (Lockwood et al., 1989). Prior to this case, all chromosomally unbalanced children involving this translocation had resulted from tertiary trisomy, and carried both normal chromosomes plus the derivative chromosome 22. This child, however, had inherited the two translocated chromosomes as well as an extra derivative chromosome 22. Although the authors concluded that this child was the result of nondisjunction for the derivative 22, a crossover in the interstitial segment followed by 3:1 segregation could well have produced this karyotype.

Indeed, such a karyotype was noted in the sperm of the t(1;11) carrier (this report).

iv. Potential bias in the segregation ratio for subtle translocations

A major criterion for studying sperm chromosomes from males heterozygous for reciprocal translocations is that the translocation must provide rearranged chromosomes that can be readily identified. Translocations involving exchange of similarly-sized segments without distinctive bands are difficult to study. Good banding technique is then needed to differentiate between normal and translocated chromosomes. An example of this is the t(2;5) translocation studied by Templado et al. (1988). It could not be determined whether the sperm chromosome complement was the result of alternate or adjacent I segregation in 44% of the karyotypes, since the translocated segments were of similar size and banding patterns. Of the two translocations studied here, the translocated chromosomes from t(1;11) were very easy to differentiate from the normal chromosomes, while the rearranged chromosomes from t(11;17) were more difficult to differentiate from the corresponding normal chromosomes. Hence, for t(11;17), karyotypes with excellent banding quality were necessary. Even so, there is a possibility that adjacent II segregation is over-represented, as the normal chromosomes and their derived counterparts could be directly compared to see subtle size

differences. Hence, the need for good chromosome bands was somewhat reduced for karyotypes resulting from adjacent II segregation as compared to karyotypes resulting from alternate and adjacent I segregations.

v. Pattern of segregation ratios

Each reciprocal translocation has a unique pattern of meiotic segregations. Nonetheless, certain characteristics are observed in all male carriers. Firstly, alternate and adjacent I disjunctions are always seen and do account for the majority of the sperm produced. Secondly, adjacent II and 3:1 segregations are not observed in every reciprocal translocation carrier and when seen, are responsible for a wide variation in the proportion of the unbalanced sperm. Considering that alternate and adjacent I segregations involve disjunction of homologous centromeres, it is not surprising to see these segregations more frequently. On the contrary, adjacent II, 3:1, and 4:0 segregations require nondisjunction of homologous centromeres. Pellestor et al. (1989) suggest that such nondisjunction during meiosis requires more energy and that these segregations are therefore less likely to occur.

vi. Animal studies

The only other mammal that has had meiotic segregation products studied as thoroughly as those of humans is the Chinese hamster. To date, eight different reciprocal translocations have been examined in these

hamsters (Sonta and Kitayama, 1987). Alternate, adjacent I, adjacent II and 3:1 segregations were found in 38.3% to 48.0%, 27.3% to 36.0%, 6.0% to 19.4%, and 4.1% to 22.0%, respectively, of metaphase II (MII) cells. Comparison of these frequencies to those obtained for human reciprocal translocation carriers reveal many similarities in the meiotic segregation ratios. Again, alternate and adjacent I segregations produced the majority of MII cells, while adjacent II and 3:1 segregations were seen less frequently and more variably. This suggests that quadrivalents in mammals behave in similar fashions.

vii. Factors predisposing the quadrivalents to adjacent II and 3:1 segregations

Various researchers have attempted to determine the factors that influence the frequencies of the various meiotic segregations in males heterozygous for reciprocal translocations. Based on data from human liveborns, Lindenbaum and Bobrow (1975) suggested that reciprocal translocations with short interstitial segments and/or the presence of an acrocentric chromosome are predisposed to 3:1 segregation. Conceivably, any factor that would result in one chromosome of the quadrivalent with no chiasmata, subsequently leading to the formation of a trivalent/ univalent combination, would increase the frequency of 3:1 segregation (Lindenbaum and Bobrow, 1975, Chandley, 1988). It has been suggested that human reciprocal translocations with short interstitial segments

that prevent crossovers in this region, and involve chromosome 9 and/or acrocentrics are predisposed to adjacent II segregation (Jalbert and Sele, 1979). Meiotic studies in mice have failed to demonstrate any relationship between meiotic configurations and the frequency of adjacent II segregation (de Boer, 1976). Using hamsters heterozygous for reciprocal translocations, Sonta and Kitayama (1987) were able to demonstrate only a relationship between the length of the chromosomes involved in the translocation and 3:1 segregation. No relationship between any factors and adjacent II segregation could be established.

A similar analysis was attempted using the data on human sperm chromosome complements from reciprocal translocation carriers. The frequencies of the various meiotic segregations were used only if a minimum of 75 sperm karyotypes had been studied. This criterion was used to ensure that skewed values caused by small sample sizes were excluded from the analysis. Various factors such as the length of interstitial segments, involvement of certain chromosomes, and length of translocated segments were compared with the frequency of adjacent II and 3:1 segregations.

Jalbert and Sele (1979) proposed that short interstitial segments and the involvement of chromosome 9 and of acrocentrics in the translocation cause an increased frequency of adjacent II segregation. However,

no correlation between these two factors and adjacent II segregation could be detected in sperm chromosome complements. Sonta and Kitayama (1987) were also not able to show any relationship between these factors and the frequency of adjacent II segregation in hamster MII spermatocytes.

Factors proposed by Lindenbaum and Bobrow (1975), namely the involvement of acrocentrics and the decreased length of interstitial segments, were not found to be correlated to the frequency of 3:1 segregation. Factors predisposing the quadrivalent to trivalent/univalent configurations, such as short pairing arms or the presence of small chromosomes, again were not found to be correlated to the frequency of 3:1 segregation. In the hamster, Sonta and Kitayama (1987) noted a relationship between the frequency of 3:1 segregation and the length of the chromosomes involved in the translocation. It is not clear what the authors meant by "the length of the chromosomes involved in the translocation.". However, the full length of the two chromosomes involved in the translocation and the length of the translocated segments were analyzed using the human data, but no relationship was found in sperm chromosome complements.

viii. Comparison of meiotic configuration and sperm karyotypes

Comparison of meiotic configurations with the actual chromosomal content of sperm may demonstrate a

relationship between the two parameters. To date, only one reciprocal translocation heterozygote has had both meiotic and sperm chromosomes analyzed. The investigators noted a correspondence between the percentage of cells containing a ring quadrivalent and the frequency of 2:2 segregations, and between the percentage of cells with a chain quadrivalent and the frequency of 3:1 segregation (Templado et al., 1990). Whether this observation demonstrates an actual relationship between chain quadrivalents and 3:1 segregation or a coincidence remains to be elucidated. Chain quadrivalents are formed when a chiasma fails to occur in one of the pairing arms. Assuming that there is a correlation between chain quadrivalents and 3:1 segregation, the presence of a short pairing arm should increase the frequency of chain quadrivalents and consequently, the frequency of 3:1 segregation. However, some reciprocal translocations with short pairing regions have extremely low frequencies of 3:1 segregation, and some reciprocal translocations with long pairing regions have high frequencies of 3:1 segregation. Further studies are needed to determine the factor or combination of factors that predispose a translocation to 3:1 segregation.

ix. Meiotic segregation ratios in females

Meiotic segregation ratios have been determined for only male reciprocal translocation carriers. No equivalent studies have been conducted on female

heterozygotes, but data on liveborn progeny and studies done on mice imply that the meiotic segregation ratios in females are different from those in males.

Although the risk of producing a chromosomally unbalanced child is the same for male and female carriers (Boue and Gallano, 1984), abnormal children resulting from 3:1 and adjacent II segregation are largely maternal in origin (Lindenbaum and Bobrow, 1975, Jalbert and Sele, 1979). A possible explanation for this observation is the increased frequency of adjacent II and 3:1 segregation in maternal carriers (Pellestor et al., 1989). It is known that the frequency of nondisjunction in human oocytes (18.6%; Pellestor and Sele, 1988) is much higher than the frequency of nondisjunction in human sperm (3.9%; Martin and Rademaker, 1990). As adjacent II and 3:1 segregations involve nondisjunction of homologous centromeres, a higher proportion of these segregations may be produced during oogenesis. An alternative explanation for the preferential maternal transmission of adjacent II and 3:1 segregations is offered by Daniel et al. (1989). It has been speculated that 3:1 segregations are usually the result of chain configurations (Templado et al., 1990), and it is known that a preponderance of chain configurations are often associated with reduced fertility in males (Chandley, 1988). Based on these observations, Daniel et al. (1989) proposed that reciprocal translocations resulting in trisomic or monosomic

liveborns are more likely to be maternal than paternal in origin.

Studies of male and female mice carrying the same translocations revealed that female heterozygotes produced a higher frequency of 3:1 segregation than male heterozygotes (Oshimura and Takagi, 1975). This conclusion was based on chromosomal analysis of both metaphase II germ cells and postimplantation embryos. Hence, it is highly probable that male and female carriers of similar reciprocal translocations would produce different meiotic segregation patterns.

It is extremely difficult to study human oocytes because of reduced accessibility and the limited number of oocytes available. While it is difficult to obtain oocytes from normal women, it will be even more difficult to obtain oocytes from female reciprocal translocation carriers. Until equivalent detailed studies are conducted on female heterozygotes, caution should be exercised when extrapolating the risk of chromosomally unbalanced gametes from male translocation carriers to female heterozygotes.

B. Unbalanced Chromosome Complements

The percentages of chromosomally unbalanced sperm related to the translocation are 61.4% and 66.8% for t(11;17) and t(1;11), respectively. These figures fall within the range observed for the other twenty-one reciprocal translocations (19 to 77%), but are greater than the mean value of 52.4% (See Table 12). The more

extreme values in the range of frequencies for unbalanced sperm are from those carriers in studies with a small sample size, where sampling error is more likely to occur. Thus, if only those studies with more than 75 karyotypes are considered, the range is much narrower: 49% to 66%, with a mean of 58.5%. Using these figures, the percentage of unbalanced sperm for the t(11;17) carrier (61.4%) is not significantly greater than the mean, and for the t(1;11) heterozygote (66.8%), the frequency falls just outside of the range. When the results from the t(11;17) and t(1;11) carriers are incorporated into the literature data on translocation carriers, the mean frequency of unbalanced sperm increases to 53.4% overall, and to 59.3% for those studies with an adequate sample size.

i. Higher incidence of unbalanced sperm than unbalanced fetuses

The frequency of chromosomally unbalanced sperm in reciprocal translocation heterozygotes (19 to 77%) is always greater than the frequency of chromosomally unbalanced fetuses for such carriers (11.66%) (Boue and Gallano, 1984). If all methods of selection against chromosomally unbalanced gametes or zygotes are postzygotic, then the frequency of unbalanced sperm probably reflects the true frequency of unbalanced conceptuses. If so, chromosomally unbalanced conceptuses are conceived, but a large proportion is lost during early embryonic development. A study that lends credence to

this view was reported by Martin (1984) on t(11;22)(q23;q11), the most common reciprocal translocation to be observed in humans. Segregations other than the one(s) known to produce chromosomally unbalanced children were seen in the sperm of these reciprocal translocation carriers. For example, 3:1 segregation producing tertiary trisomy is the only segregation observed among the chromosomally unbalanced progeny of t(11;22) carriers. Studies on sperm chromosomes of a t(11;22) heterozygote demonstrated that all 2:2 segregations, as well as 3:1 segregations, are produced (Martin, 1984). Hence, appearance of only one, or sometimes two segregations at birth is not related to the production of unbalanced gametes but to the degree of imbalance in such gametes and the ensuing viability of the embryos.

ii. Higher frequency of unbalanced sperm in reciprocal than in Robertsonian translocations carriers

It is interesting to note that the frequency of chromosomally unbalanced sperm observed in Robertsonian translocation carriers is considerably lower than the frequency of chromosomally unbalanced sperm in reciprocal translocation heterozygotes. To date, sperm chromosome complements have been analyzed for six men heterozygous for Robertsonian translocations (t(14;21) Balkan and Martin, 1983b; t(13;14) Pellestor et al., 1987; t(13;14)

Martin, 1988b; t(13;15) Pellestor, 1990; t(15;22) and t(21;22) Syme and Martin, personal communication). The frequency of chromosomally unbalanced sperm related to the translocation was found to range from 3.7 to 27.7%, with a mean of 11.5%.

During meiosis, the chromosomes involved in the Robertsonian translocation pair to form a trivalent which can exist in two different configurations, cis and trans. The cis configuration, which is predisposed to alternate segregation, is the predominate form found in the germ cells of Robertsonian translocation heterozygotes, whereas the trans configuration, which is predisposed to adjacent segregation, is the rarer form (Pellestor, 1990). With the predisposition of the cis configuration leading to the formation of balanced gametes, the proportion of chromosomally unbalanced gametes in Robertsonian translocation carriers is less than that observed in reciprocal translocation carriers. Furthermore, the breakpoints in Robertsonian translocations are always pericentric, resulting in uniform meiotic configurations. In contrast, the breakpoints in reciprocal translocations are variable, resulting in a variety of meiotic configurations. Based on this line of thought, Pellestor (1990) proposes that heterogeneity in the meiotic configurations of reciprocal translocations results in a wide variability in the percentage of chromosomally unbalanced sperm in reciprocal translocation carriers,

while the homogeneity of the meiotic configurations in Robertsonian translocations results in a narrower range of chromosomally unbalanced sperm in Robertsonian translocation carriers.

iii. Ascertainment bias

The method used to ascertain reciprocal translocation carriers could potentially influence the frequency of chromosomally unbalanced sperm. It is known from prenatal diagnoses that reciprocal translocation carriers ascertained through the birth of a chromosomally unbalanced child have a greater risk for chromosomally unbalanced liveborns than reciprocal translocation carriers ascertained through other mechanisms (Boue and Gallano, 1984). However, the increased risk for chromosomally abnormal children is likely correlated with the degree of imbalance associated with the translocation, and not with the percentage of chromosomally unbalanced gametes.

Male carriers whose sperm chromosomes have been analyzed were ascertained in one of four ways: a family history of chromosomally unbalanced children, a family history of multiple spontaneous abortions, infertility, or purely by chance either through amniocentesis for advanced maternal age or through sperm donation for other reasons. A statistical analysis on the frequency of chromosomally unbalanced sperm from studies using the four possible methods of ascertainment revealed no differences in the

means. (ANOVA, $F=0.24 < F_{.10}(3,16)$; Johnson and Bhattacharyya, 1985, p.473). Hence, the frequency of chromosomally unbalanced sperm is not dependent upon the method of ascertainment.

C. Abnormalities unrelated to the translocation

i. Numerical abnormalities

Numerical abnormalities unrelated to the translocation accounted for 6.9% and 5.6% of the sperm karyotypes analyzed for the t(11;17) and t(1;11) carriers, respectively. These values fall within the range observed for normal men, 0% to 10%, and both are within one standard deviation of the mean, 4.7% ($\pm$ 2.9%) (Martin et al., 1987).

Although, in normal control donors, there was no significant relationship between the frequency of numerical abnormalities and age, the frequency of hyperhaploid complements was found to be negatively correlated with age (Martin and Rademaker, 1987): younger men had higher frequencies of hyperhaploid sperm than older men. Comparison of the frequency of hyperhaploid sperm found in the t(11;17) heterozygote with rates found in other men studied in the same age category (20 to 24 years) and in the same laboratory, indicated that the percentage of hyperhaploid sperm observed in t(11;17) (1.0%) is just outside the established range (1.5% to 5.3%) and is below the mean (3.7%) (Martin and Rademaker, 1987). In the t(1;11) carrier, the frequency of

hyperhaploidy (0.35%) falls within the range (0% to 2.8%) observed in other men studied in the same laboratory in his age category (25 to 29 years), and is just slightly lower than the mean of 0.8% (Martin and Rademaker, 1987).

Breakdown of numerical abnormalities into frequencies of hypohaploidy and hyperhaploidy showed an excess of hypohaploid complements. For the t(11;17) carrier, 5.9% were hypohaploid, 1.0% were double aneuploid, and 0% were hyperhaploid. For the t(1;11) heterozygote, 5.2% were hypohaploid and 0.35% were hyperhaploid. An excess of hypohaploidy is a common observation in sperm chromosome complements (Martin et al., 1987, Pellestor et al., 1989, Templado et al., 1990, Martin and Rademaker, 1990) and in karyotypes of human lymphocytes (Ford et al., 1988).

Although the observed excess of hypohaploidy is often attributed to technical artifacts, such as chromosome loss or scattering during fixation, Ford et al. (1988) demonstrated that anaphase lagging is a major mechanism of chromosome loss in human lymphocyte culture. Anaphase lagging of chromosomes or chromatids, followed by elimination through micronuclei, would result in the production of two hypoploid cells or one normal and one hypoploid cell, respectively. Hence, the number of cells gaining a chromosome would not necessarily equal the number of cells losing a chromosome. From studies of human lymphocytes, anaphase lagging was found to occur in 2.43% of cells (Ford et al., 1988).

The majority of chromosomes lost in hypohaploid sperm complements are the smaller chromosomes (Martin and Rademaker, 1990). For both the t(11;17) and t(1;11) heterozygotes, loss of smaller chromosomes, chromosomes 16 to 22, was observed in half of the hypohaploid karyotypes. Preferential loss of the smaller chromosomes could either be due to technical artifacts or anaphase lagging. Physically speaking, scattering or loss of a smaller chromosome during fixation is more likely to occur than a similar loss of a larger chromosome. Likewise, anaphase lagging may preferentially affect the smaller chromosomes. Chromosome displacement from the metaphase plate, which may occur as a step preceding anaphase lagging, was found to favour the displacement of smaller chromosomes over larger chromosomes (Ford and Lester, 1982, Ford et al., 1988).

It is difficult to determine what portion of the hypohaploid complements is the result of anaphase lagging or technical artifacts. By doubling the frequency of hyperhaploidy, a conservative estimate of the aneuploidy rate can be calculated (Martin et al., 1987). Conservative estimates of the aneuploidy rate for t(11;17) and t(1;11) are 2.0% and 0.7%, respectively, and is within the range (0% to 10.6%) observed in normal men (Martin et al., 1987). While this figure may slightly underestimate the true aneuploidy frequency, it provides an unbiased method for comparison. Recently, a new technique, in situ

hybridization, has been developed to detect aneuploidy in human sperm nuclei (West et al., 1989, Guttenbach and Schmid, 1990). This technique may overcome the problem of "losing" chromosomes and hence, could reflect the true rate of aneuploidy in human sperm.

Hyperhaploidy was observed only for chromosome 1, chromosome 21, and chromosome Y in the sperm karyotypes from the t(11;17) and t(1;11) carriers. However, the analysis of 6,821 sperm chromosome complements obtained from 98 men revealed that hyperhaploidy was observed for sex chromosomes and for representatives of all Denver groups (Martin and Rademaker, 1990). Hence, it appears that all chromosomes are subject to nondisjunction.

ii. Structural abnormalities

Structural anomalies unrelated to the translocation were noted in 19.8% and 9.2% of the sperm chromosome complements analyzed from the t(11;17) and t(1;11) carriers, respectively. Studies of sperm chromosomes from normal men in the same laboratory have revealed a range of 0% to 23.1%, with a mean of 6.2% (±6.0%) for structural abnormalities (Martin et al., 1987). For both translocations, t(11;17) and t(1;11), the percentages of structural abnormalities are higher than the mean, but still fall within the range observed for normal men.

The frequency of structural abnormalities observed in human sperm from normal control donors was found to be positively correlated to the age of men (Martin and

Rademaker, 1987): younger men had lower frequencies of structural abnormalities while older men had higher frequencies of structural anomalies. The frequency of structural abnormalities for the t(11;17) carrier (19.8%) was higher than any incidence observed for men aged 20 to 24 years (range 1.3% to 5.8%, mean 2.8%) (Martin and Rademaker, 1987). For the t(1;11) heterozygote, the frequency of structural anomalies (9.2%) was also higher than any rate observed for men 25 to 29 years of age (range 0% to 5.6%, mean 2.2%) (Martin and Rademaker, 1987). It is surprising to see such high frequencies of structural anomalies in young men, but perhaps these rates reflect the huge variability seen between individual donors or possible exposures to unknown clastogens.

A wide range of structural abnormalities affecting chromatids and chromosomes was observed. For both reciprocal translocation carriers, sperm chromosome complements with extensive breaks and/or rearrangements, where the chromosomal content could not be thoroughly identified accounted for approximately half of the karyotypes with structural anomalies. The next most prevalent groups of structural abnormalities involved chromosome aberrations (42.5% and 45.3%), and followed by chromatid aberrations (12.5% and 5.7%), for t(11;17) and t(1;11), respectively.

The detection of certain structural abnormalities such as small deletions, translocations involving

similarly-sized segments, and inversions not involving the centromere, are dependent upon the quality of chromosome banding. Hence, the frequency of structural anomalies may be underestimated. Conversely, there have been suggestions that some of the structural abnormalities seen are the result of the experimental procedures used and consequently the frequency of structural abnormalities is exaggerated. First, the use of TEST yolk buffer for 48 hours to capacitate the sperm and to enhance fertilization has been shown to increase the frequency of structural abnormalities (Martin et al., 1990c). Although TEST yolk buffer was used to obtain sperm chromosomes from these two reciprocal translocation carriers, the length of use did not exceed 24 hours. Nonetheless, it is possible that the use of this medium has slightly increased the frequency of structural abnormalities. Secondly, there has been speculation that some structural abnormalities, especially those involving single chromatids, are the result of sperm chromosomes replicating in a foreign environment (Martin et al., 1987, Brandriff et al., 1988). However, chromatid aberrations account for only approximately 10% of all structural abnormalities observed in the two translocation heterozygotes. Brandriff et al. (1988) argue against this speculation, using three lines of evidence. Firstly, extensive variability exists among donors (Martin et al., 1987, Brandriff et al., 1988). If the experimental technique were inducing the structural abnormalities,

variability between donors should not be pronounced. Secondly, the frequency of structural abnormalities for an individual donor remains the same over time (Brandriff et al., 1985b). If the breaks were experimentally induced, one would expect random occurrence rather than the observed intra-donor consistency. Thirdly, there is no evidence that hamster oocytes providing the substrates for replication of more than one human sperm became depleted and caused an increased frequency of structural aberrations. If the hamster oocyte milieu was responsible for inducing structural aberrations, the presence of more than one human sperm per oocyte should result in an increased likelihood for structural anomalies. Nonetheless, further studies are required to rule out the possibility of a cross-species interaction in the production of structurally abnormal sperm (Martin et al., 1987).

Structural abnormalities are not usually seen in liveborn offspring unless they involve a balanced rearrangement or a minimal loss/gain of chromosomal material. Sperm with extensive chromosomal damage appear not to result in viable conceptuses because they have never been reported in any clinically recognizable pregnancy.

D. Sex Ratios

For the t(11;17) and the t(1;11) heterozygotes, the frequency of X-bearing sperm to Y-bearing sperm was 51:49 and 52:48, respectively. As expected, neither was significantly different from a 1:1 ratio. No other individual studied by sperm chromosome analysis has shown a significant deviation of X- to Y-bearing sperm from the expected 1:1 ratio. When Martin (1990) compiled all the data on sperm chromosome complements analyzed in her laboratory, there was a minimal but statistically significant excess of X-bearing sperm. She speculates that the human sperm/hamster oocyte fusion technique may preferentially favour fertilization by X-bearing sperm over Y-bearing sperm (Martin, 1990).

E. Sperm Selection

The female genital tract allows only a subpopulation of sperm to reach the site of fertilization (Mortimer, 1979, Nestor and Handel, 1984, Redi et al., 1984). Non-motile, morphologically abnormal sperm are at a disadvantage, while highly motile, morphologically normal sperm are at an advantage for bypassing the cervix. While the selected sperm are physiologically superior, several recent studies have revealed that the genetic content of these sperm is not significantly different from that of unselected sperm (Foldesy et al., 1984, Redi et al., 1984, Brandriff et al., 1986b, Estop et al., 1988). Redi et al. (1984) studied the distribution of Feulgen-DNA content of

sperm in the female genital tract and found no changes in the distribution along the tract, implying that both balanced and unbalanced sperm were capable of fertilization. Foldesy et al. (1984) did an unique experiment where the female reproductive tract was bypassed by placing the sperm directly into the oviduct. The survival of embryos derived from this technique was compared to that of embryos obtained by natural fertilization. No significant differences were observed in the embryo survival levels, implying that sperm capable of reaching the site of fertilization were not genetically superior to unselected sperm.

Likewise, direct examination of the chromosomal content of highly motile sperm has shown no significant difference from the chromosomal content of unselected sperm. Human sperm selected for motility by the swim-up procedure were studied by the human sperm/hamster oocyte fusion technique (Brandriff et al., 1986b). The frequency of chromosomal abnormalities in these selected sperm was not different from that found in the less-motile sperm. A similar study was conducted using mouse sperm (Estop et al., 1988). Again, no statistical difference was detected between the selected, motile sperm and the unselected sperm.

Detection of embryos with gross genomic imbalances implies that chromosomally unbalanced sperm are capable of fertilizing oocytes, and that elimination must occur post-

zygotically. Prior to the study by Epstein and Travis (1979), it was unclear if nullisomic gametes were incapable of fertilizing oocytes or if ensuing embryos were eliminated before the implantation stage. Studies of day 3 embryos were conducted and monosomies for chromosomes 1, 12 and 19 were detected. However, by day 4, embryos monosomic for chromosome 1 and 19 had reduced viability, demonstrating that these embryos are produced and then are selectively eliminated during the preimplantation stage.

A similar study was conducted in Chinese hamsters to determine if gametic and/or zygotic selection occurred (Sonta et al., 1984). The frequency of chromosomally unbalanced metaphase II cells was recorded for male hamsters heterozygous for a reciprocal translocation and was compared to the frequency of chromosomally unbalanced two-cell embryos resulting from a backcross of these males. No detectable differences in the frequencies were noted, indicating that neither gametic selection nor zygotic selection prior to the first cleavage had occurred. However, using a different reciprocal translocation, the frequency of chromosomally unbalanced one-cell embryos was significantly lower than the frequency of chromosomally unbalanced metaphase II cells (Sonta and Kaseki, 1986). The difference in frequencies was limited to embryos partially monosomic for chromosome

1, suggesting that there is a possibility that sperm selection exists.

A large body of indirect evidence suggests that there is no selection based on chromosomal content in the human sperm/hamster oocyte fusion technique. Martin and Rademaker (1988) found no significant relationship between the percentage of morphologically abnormal sperm in the semen sample and the percentage of chromosomally abnormal sperm. This suggests that morphologically abnormal sperm are not specifically unbalanced in chromosomal content. Also, men who produced a high frequency of chromosomally unbalanced gametes, such as radiotherapy patients and carriers of balanced rearrangements, did not have an increased incidence of morphologically abnormal sperm (Martin and Rademaker, 1988). The technique used for examining human sperm chromosome complements omitted selection by the female reproductive tract, allowing both motile and non-motile sperm in the vicinity of the oocytes. However, as shown by Brandriff et al. (1986b), there is no genetic difference between selected sperm and unselected sperm. Furthermore, utilization of techniques that alter the viability of human sperm such as freezing, do not appear to affect the frequency of sperm with chromosomal abnormalities.

The frequency of chromosomally unbalanced sperm in reciprocal translocation carriers ranges from 19% to 77%, which is higher than the incidence of chromosomally

unbalanced fetuses (Boue and Gallano, 1984). Either prezygotic or postzygotic selection must account for the decreased frequency of chromosomal abnormalities seen in clinically recognized pregnancies. Because the risk of producing chromosomally unbalanced offspring is the same for maternal and paternal reciprocal translocation carriers, it seems unlikely that sperm selection exist *in vivo* (Martin, 1983b). Also, based on studies conducted in mice (Epstein and Travis, 1979) and, particularly, in hamsters (Sonta *et al.*, 1984), postzygotic selection appears to be responsible for eliminating the majority of chromosomally unbalanced embryos. However, for one of three reciprocal translocations studied in the Chinese hamster, a possibility for sperm selection did exist. To date, in all reciprocal translocations studied, except the t(1;11) carrier (this report), adjacent II segregation has produced the two complementary gametes in a 1:1 ratio. In the t(1;11) carrier, the number of sperm karyotypes missing both the normal and derivative forms of chromosome 1 was considerably less than the number of sperm karyotypes missing both the normal and derivative forms of chromosome 11. Perhaps, for the t(1;11) carrier, sperm nullisomic for chromosome 1 are at a disadvantage during fertilization and thereby, are largely eliminated prezygotically.

Because sperm selection does not appear to play a major role either *in vivo* or *in vitro*, the human

sperm/hamster oocyte fusion technique should reflect the true frequencies of chromosomally unbalanced sperm *in vivo*. Further studies are needed to determine whether sperm selection does exist and if so, how it occurs. Evidence obtained to date indicates that postzygotic selection is the major mechanism for eliminating chromosomally unbalanced conceptuses (Epstein and Travis, 1979, Sonta *et al.*, 1984). However, prezygotic selection may play a secondary role in the elimination of certain chromosomally unbalanced sperm (Sonta and Kaseki, 1986).

F. Interchromosomal Effect

Individuals heterozygous for structural rearrangements are thought to be predisposed to nondisjunction of normal chromosomes and consequently are at an increased risk for having aneuploid children (Aurias *et al.*, 1978, Lindenbaum *et al.*, 1985). However, prior studies examining the theory of an interchromosomal effect have disclosed conflicting evidence. If individuals carrying structural rearrangements are indeed at an increased risk for producing monosomic and trisomic children, the frequency of aneuploidy unrelated to the translocation will also be increased in the gametes. Hence, cytogenetic analysis of human sperm could provide evidence for or against the theory of an interchromosomal effect.

For the t(11;17) and t(1;11) heterozygotes, 202 and 575 sperm karyotypes, respectively, were obtained. These

sample sizes met the criterion for detecting a tripling and a doubling, respectively, in the frequency of aneuploidy. However, both reciprocal translocation carriers had an aneuploidy frequency that was lower than that observed in normal men. Thus, these studies failed to provide any evidence for an interchromosomal effect.

Of the 22 reciprocal translocation carriers studied to date (See Table 13), eight have had a minimum of 160 sperm analyzed (Brandriff et al., 1986a, Martin, 1988a, Martin et al., 1990b, Martin, personal communication, this report) and only one has had a minimum of 500 sperm analyzed (this report). None of these nine men showed a significant increase in the frequency of aneuploidy. Only one man, a carrier for two different reciprocal translocations, showed an increased frequency of numerical abnormalities (70%). While this figure is based on a sample size of only 23 sperm karyotypes, the frequency of aneuploidy is considerably higher than that observed in normal men. It is possible that the presence of one translocation may be insufficient to disrupt normal meiotic pairing, while the presence of a second translocation may be sufficient to cause nondisjunction of normal chromosomes.

Recently, Martin and Rademaker (1990) have published an updated conservative estimate for the aneuploidy frequency (1.5%). Although this figure is based on studies of 83 normal men and 15 men heterozygous for

Table 13: Frequency of unrelated numerical and structural abnormalities observed in 22 men heterozygous for 23 reciprocal translocations.

Translocation Observed	# Spreads	Numerical Observed	Numerical Estimate+	Structural
(11;22)[1]	13	7.6	0.0	7.6
(6;14)[2]	19	0.0	0.0	10.5
(7;14)[3]	19	0.0	0.0	0.0
(5;11)[4a]	23	69.6*	60.9*	13.0
(7;14)[4a]	23	69.6*	60.9*	13.0
(5;18)[2]	32	15.6	12.5	9.4
(5;13)[5]	57	10.5	0.0	0.0
(4;17)[5]	60	13.3	3.3	0.0
(2;5)[6]	75	12.0	8.0	4.0
(9;18)[5]	82	8.5	4.9	2.4
(6;7)[5]	84	8.3	0.0	1.2
(1;2)[7]	105	14.3	7.6	14.3
(12;20)[8]	113	11.5	1.8	6.2
(15;22)[9]	152	3.9	0.0	8.6
(4;6)[3]	164	9.1	2.4	18.9
(9;10)[10]	171	8.2	5.8	15.2
(16;19)[9]	178	6.0	0.0	9.8
(3;16)[11]	201	1.5	1.0	5.6
(11;17)[12]	202	6.9	2.0	19.8
(2;9)[3]	217	4.3	0.0	21.7
(8;15)[11]	226	2.6	2.6	2.7
(3;11)[9]	268	3.4	0.0	8.2
(1;11)[12]	575	5.6	0.7	9.2
Normal Range[13]		4.7(±2.9)	2.4	6.2(±6.0)

+This figure represents the conservative estimate of aneuploidy, which was derived by doubling the frequency of hyperhaploidy.
*Significant increase in the frequency of unrelated numerical abnormalities.
[a]Both translocations are present in one male.
[1]Martin, 1984
[2]Balkan and Martin, 1983a
[3]Martin et al., 1990b
[4]Burns et al., 1986
[5]Pellestor et al., 1989
[6]Templado et al., 1988
[7]Templado et al., 1990
[8]Martin et al., 1990a
[9]Martin, personal communication
[10]Martin, 1988a
[11]Brandriff et al., 1986a
[12]Spriggs, this report
[13]Martin et al., 1987

structural rearrangements, results obtained from the 15 carriers for constitutional chromosomal abnormalities were pooled with those obtained from normal men only after statistical analysis showed no significant differences in the aneuploidy rates. In other words, these men showed no evidence for an interchromosomal effect, and thus should not skew the frequency of aneuploidy seen in normal men. Using this updated aneuploidy frequency of 1.5% (Martin and Rademaker, 1990), a significance level of 0.05, and a power level of 0.90, the sample sizes needed to detect a tripling and a doubling, respectively, in the aneuploidy frequency are calculated to be 241 and 778 karyotypes. These sample sizes are considerably larger than those needed for the previous aneuploidy frequency of 2.4% (Martin et al., 1987). It may be quite some time before the existence of an interchromosomal effect is verified or disproven using this updated aneuploidy frequency. As the technique is extremely tedious and time-consuming, it is not a trivial undertaking to analyze more than 200 karyotypes.

Sperm chromosomal complements from men carrying constitutional chromosomal abnormalities other than reciprocal translocations have been studied (rob(14;21) Balkan and Martin, 1983b; inv(3)(p11q11) Balkan et al., 1983; inv(7)(q11q22) Martin, 1986; 47,XY,+mar Martin et al., 1986; rob(13;14) Pellestor et al., 1987; 47,XYY Benet and Martin, 1988, rob(13;14) Martin, 1988b; rob(13;15)

Pellestor, 1990; rob(15;22) and rob(21;22) Syme and Martin, personal communication). Of the eleven men studied, only one male, carrying an accessory marker, displayed a possible interchromosomal effect. However, only 31 sperm karyotypes were analyzed for this individual, and it is conceivable that the conservative estimate of aneuploidy (16%) obtained was biased by the small sample size.

Researchers studying chiasma frequency and chiasma distribution in meiotic chromosomes of individuals heterozygous for structural rearrangements, have noticed a change, not only in the rearranged chromosomes, but in other unaffected chromosomes (Laurie et al., 1984, Laurie et al., 1985). How this interchromosomal effect for chiasmata could be related to the interchromosomal effect for aneuploidy is not clear.

Meiotic studies on chromosomally normal males have revealed that chiasma frequency and chiasma distribution is relatively homogeneous for those chromosomes examined in detail. Due to the lack of variability in the chiasma frequency or chiasma distribution among normal men, similar studies on men carrying structural rearrangements can easily detect any differences in this chiasma frequency and/or distribution (Laurie et al., 1984). Detailed meiotic studies have been conducted on three reciprocal translocation carriers, t(1;22) (Palmer and Hulten, 1983), t(Y;10) (Laurie et al., 1984), and t(9;10)

(Laurie et al., 1984, Laurie et al., 1985). An increase in the chiasma frequency was detected for all three reciprocal translocation carriers. For two heterozygotes, t(1;22) and t(Y;10), the increase was localized to the quadrivalent. For the third carrier, t(9;10), the chiasma frequencies were not only increased for the quadrivalent, but for chromosomes 1 and 2. Meiotic chromosomes have been examined in other reciprocal translocation carriers, and to date it appears that the chiasma frequencies can remain unaltered, be increased or be decreased, and that the chiasma distribution can be the same or different from that in normal men (Laurie et al., 1984).

The relationship between an interchromosomal effect for chiasma frequency and an interchromosomal effect for aneuploidy has not been directly examined. However, based on Grell's (1971) hypothesis, it is thought that non-crossover chromosomes enter into a distributive pool, and subsequently undergo distributive pairing. If the presence of a structural rearrangement results in an overall decrease in chiasma frequency, then the likelihood of chromosomes with no crossovers is increased. Consequently, these chromosomes enter the distributive pool, resulting in an increased probability for nondisjunction. Concurrent studies of meiotic and sperm chromosomes may reveal the relationship between the two types of interchromosomal effects.

Both increases and decreases in the chiasma frequencies have also been noted in grasshoppers heterozygous for reciprocal translocations (Arana et al., 1990). However, Arana et al. (1990) discovered that the same translocation in different genetic backgrounds produced different results. For one strain, the mean chiasma frequency was increased in the heterozygote, and for the remaining three, the mean chiasma frequencies were decreased in the heterozygotes. The investigators suggested that the interchromosomal effect exerted by a certain rearrangement was dependent on the genetic background. If different genetic factors cause different effects on chiasma frequency, then perhaps only some individuals heterozygous for structural rearrangements will display an increased frequency of nondisjunction. It is possible that the reciprocal translocation carriers who have had at least 160 karyotypes analyzed were not genetically predisposed to an interchromosomal effect. Hence, more reciprocal translocation carriers need to be studied to detect those with an increased risk for an interchromosomal effect.

Most sperm chromosome analysis conducted on individuals heterozygous for reciprocal translocations have failed to provide support for the theory of an interchromosomal effect. The only possible exception is the double reciprocal translocation carrier (Burns et al., 1986). Meiotic studies on reciprocal translocation

carriers have demonstrated an interchromosomal effect on the chiasma frequency for one individual (Laurie et al., 1984, Laurie et al., 1985). However, the relationship between an interchromosomal effect for chiasma frequency and an interchromosomal effect for aneuploidy has not yet been established. Furthermore, studies conducted on grasshoppers suggest a possible genetic influence on the presence of an interchromosomal effect (Arana, 1990). Hence, sperm from more reciprocal translocation carriers need to be analyzed before the theory of an interchromosomal effect is discarded.

VI. CONCLUSION

Direct cytogenetic examination of the sperm chromosomal content was conducted for two reciprocal translocation heterozygotes, t(11;17) and t(1;11). The analysis revealed the frequencies of unbalanced sperm complements related to the translocation to be 61.4% and 66.8%, respectively. While all segregations, with the exception of 4:0 segregation, were observed, adjacent I segregation was responsible for the majority of the chromosomally unbalanced sperm. Adjacent II segregation was the next most prevalent segregation observed, followed by 3:1 segregation. Although certain characteristics were common to both reciprocal translocation carriers, the actual meiotic segregation frequencies were unique. It is not yet possible to determine which factors cause the wide variations observed in meiotic segregation ratios.

The frequency of chromosomal abnormalities unrelated to the translocation, and particularly the conservative estimate for the frequency of aneuploidy, are within the range observed for normal men (Martin et al., 1987). Prior to this study, the largest sample size conducted on a single reciprocal translocation carrier was 268 karyotypes (Martin, personal communication). Thus, the 575 sperm chromosome complements analyzed for the t(1;11) heterozygote, sufficient to detect a doubling in the conservative estimate of aneuploidy frequency, is now the largest sample size collected on a single reciprocal

translocation carrier. However, an increase in the conservative estimate for the rate of aneuploidy was noted in neither the t(1;11) nor t(11;17) carriers, implying that an interchromosomal effect is not present in either reciprocal translocation carrier. Of the 22 reciprocal translocation carriers studied to date, only one, a double translocation heterozygote, had a significant increase in the aneuploidy frequency. Hence, it appears that single reciprocal translocation carriers are not at an increased risk for producing an aneuploid child.

The number of reciprocal translocations possible at the 293 band resolution is over 40,000 (De Arce et al., 1986). Since fewer than 3% have been reported, the possibility of encountering a new reciprocal translocation is immense. As the reproductive risk for each reciprocal translocation carrier is unique, genetic counselling is rendered more difficult. Sperm chromosome analysis conducted on reciprocal translocation heterozygotes has revealed that, on average, approximately half of the sperm is chromosomally unbalanced, with a major proportion due to adjacent I segregation. While it is not yet possible to predict the frequencies of the various segregations for each reciprocal translocation carrier, further studies may make this goal attainable. Furthermore, with a lack of concrete evidence for an interchromosomal effect, it seems unnecessary to counsel translocation carriers on increased risks of having an aneuploid child.

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VIII. APPENDICES

A. Media and Solutions

BWW stock solution

	grams/litre
NaCl	5.540
KCl	0.356
$CaCl_2$ (pellets)	0.189
KH_2PO_4	0.162
$MgSO_4.7H_2O$	0.294
Pyruvic Acid, Na Salt	0.028
Dextrose	1.000
	millilitres/litre
Antibiotic stock solution	1.0
0.5% Phenol Red	0.5
Acid Hepes	9.5
Base Hepes	10.5
Distilled H_2O	to 1000.0 ml

Maintain solution at 4°C for a maximum of two weeks.

BWW Working Solution

$NaHCO_3$	0.2106 g
DL-Lactic Acid, Na Salt (60% syrup)	0.37 ml
Human Serum Albumin (Fraction V)	0.5 g
BWW Stock Solution	100 ml

Dissolve crystals, mix, and filter-sterilize through a cellulose acetate/nitrate membrane, pore size 0.22 um.

Antibiotic Stock Solution for BWW

Penicillin-G, Na Salt	10^5 IU/ml
Streptomycin sulfate	50 mg/ml

Freeze in 1 ml aliquots.

Acid Hepes (2M Hepes in distilled H_2O)

Hepes	47.66 g
Distilled H_2O	to 100 ml

Base Hepes (2M Hepes in 3M NaOH)

NaOH	12 g
Hepes	47.66 g
Distilled H_2O	to 100 ml

F10 Working Solution (15% Fetal Bovine Serum)

Ham's F10	42.5 ml
Fetal Bovine Serum (heat inactivated at 56°C for 30 minutes)	7.5 ml
Antibiotic Stock Solution	0.5 ml

Antibiotic Stock Solution for F10 Working Solution

Penicillin-G, Na Salt	10^5 IU/ml
Streptomycin Sulfate	5 mg/ml

Hyaluronidase Solution (0.2%)

Hyaluronidase (Type 1-S)	3 mg
BWW Working Solution	1.5 ml

Working concentration will be halved as it is added to an equivalent volume of BWW working solution.

Trypsin Solution (0.1%)

Trypsin (Type XII)	3 mg
BWW Working Solution	3 ml

Colcemid Solutions

For Afternoon Experiments (0.8 ug/ml)

Colcemid (Gibco, 10 ug/ml)	0.2 ml
F10 Working Solution	2.3 ml

For Morning Experiments (0.4 ug/ml)

Colcemid (Gibco, 10 ug/ml)	0.1 ml
F10 Working Solution	2.4 ml

Concentration in drop will be halved as it is added to an equivalent volume of F10 working solution.

Sperm Count Diluent

$NaHCO_3$	5 g
35% Formaldehyde	1 ml
0.9% NaCl	100 ml

Mix and store at 4°C.

Ackerman's Cryopreservant

Bacto Egg Yolk Enrichment - 50%	40 ml
Glycerol	30 ml
Glycine	2.0 g
Glucose	2.6 g
Sodium Citrate	2.3 g
Distilled H_2O	98.7 ml

Heat inactivate at 56°C for 30 minutes. Adjust pH and store at -20°C in aliquots of desired volume.

TEST Yolk Buffer Salt Stock

TES	2.1629 g
Tris	0.5135 g
Dextrose	0.1000 g
Streptomycin Sulfate	0.0125 g
Penicillin-G, Na Salt	0.0075 g
Distilled H_2O	to 50 ml

Freeze in 10 ml aliquots.

TEST Yolk Buffer

Fresh Hen's Yolk	2.5 ml
TEST Yolk Buffer Salt Stock	10 ml

Mix and centrifuge at ~1200xG for 10 minutes. Decant supernatant and adjust its pH with solid Tris. Store 1 ml aliquots at -20°C.

Giemsa Solid Stain (5-6% in Gurr Buffer)

Harleco Giemsa Stain	3 ml
Gurr Buffer	50 ml

Skim off oxidized surface. Make fresh daily.

Gurr Buffer

1 Gurr ® Buffer tablet (pH 6.8)

in 1000 ml distilled H_2O

Adjust pH to 4.4 and store at 4°C.

Quinacrine Dihydrochloride

Quinacrine Dihydrochloride	0.25 g
Distilled H_2O	50 ml

May be maintained at 4°C for one week. Wrap container in foil to protect from light.

B. Reagents

Bacto Egg Yolk Enrichment (50%)	Difco 3347-73-8
$CaCl_2$ (pellets)	Fisher C-614
Colcemid (10 ug/ml)	Gibco G.D. 1024
Formaldehyde	BDH B10113
D-Glucose (Dextrose)	Fisher D-16
DL-Lactic Acid, Na Salt (60% syrup)	Sigma L4263
Ethanol	
Fetal Bovine Serum (Cellect Silver)	Flow 29-161-49
Giemsa Stain	Harleco 620
Glacial Acetic Acid	Fisher A38-4
Glycerol	
Gurr ® Buffer Tablets (pH 6.8)	Hopkins and Williams 065568
Glycine	
1X Ham's F10 with L-Glutamine	Flow 12-403-54
Hepes	Sigma H3375
Human Chorionic Gonadotropin	A.P.L. Ayerst
Human Serum Albumin (Fraction V)	Sigma A2386
Hyaluronidase (Type 1-S)	Sigma 3506
KH_2PO_4	Fisher P-382
$MgSO_4.7H_2O$	Fisher M-63
NaCl	Fisher S671-500
Na Citrate	Fisher S279
$NaHCO_3$	Fisher S233
NaOH	Fisher S318B
Penicillin-G, Na Salt	Sigma P-3032
Phenol Red	EM PX0530-3

Polyvinylpyrollidone	
Pregnant Mares' Serum Gonadotropin	Sigma G4877
Pyruvic Acid, Na Salt (Type II)	Calbiochem 5510
Quinacrine Dihydrochloride	Sigma Q-0250
Streptomycin Sulfate	Sigma S-6501
TES (N-tris[Hydroxymethyl]methyl-2-aminoethanesulfonic acid])	Sigma T-1375
Tris (Trizma ® Base - Tris [Hydroxymethyl]aminomethane)	Sigma T-1503
Trypsin (Type XII)	Sigma 2884