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Cytogenetics of Human Sperm: Meiotic Segregation in Two Translocation Carriers

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SUMMARY

Meiotic segregation products were studied in sperm from two men heterozygous for the reciprocal translocations t(8;15)(p22;q21) and t(3;16)(p23;q24). A total of 226 and 201 sperm complements, respectively, were analyzed. In each translocation, 63% of complements were unbalanced, and alternate and adjacent 1 percentages were similar. The 3:1 segregation frequencies produced by the two translocations were 3.5% and 5.0%.

INTRODUCTION

An increased risk for reproductive failure is experienced by reciprocal translocation carriers. They produce chromosomally unbalanced gametes, with relative frequencies depending on segregation events at meiotic disjuction (see fig. 1). Because of the uniformity of meiotic events across species, the behavior of human translocations at meiosis has been predicted on the basis of segregation in animals and plants [1]. Segregation mechanisms also can be inferred from studies of live borns with unbalanced karyotypes [3]. These latter studies can be open to error because of small numbers and bias in selection of subjects [4]. Furthermore, each individual reciprocal translocation represents a unique situation because the frequencies of various types of unbalanced gametes depend on the chromosomes involved, the breakpoints, and the number and location of chiasmata [1]. As a result, individual translocation carriers can be given only an estimate of their reproductive risk [5].

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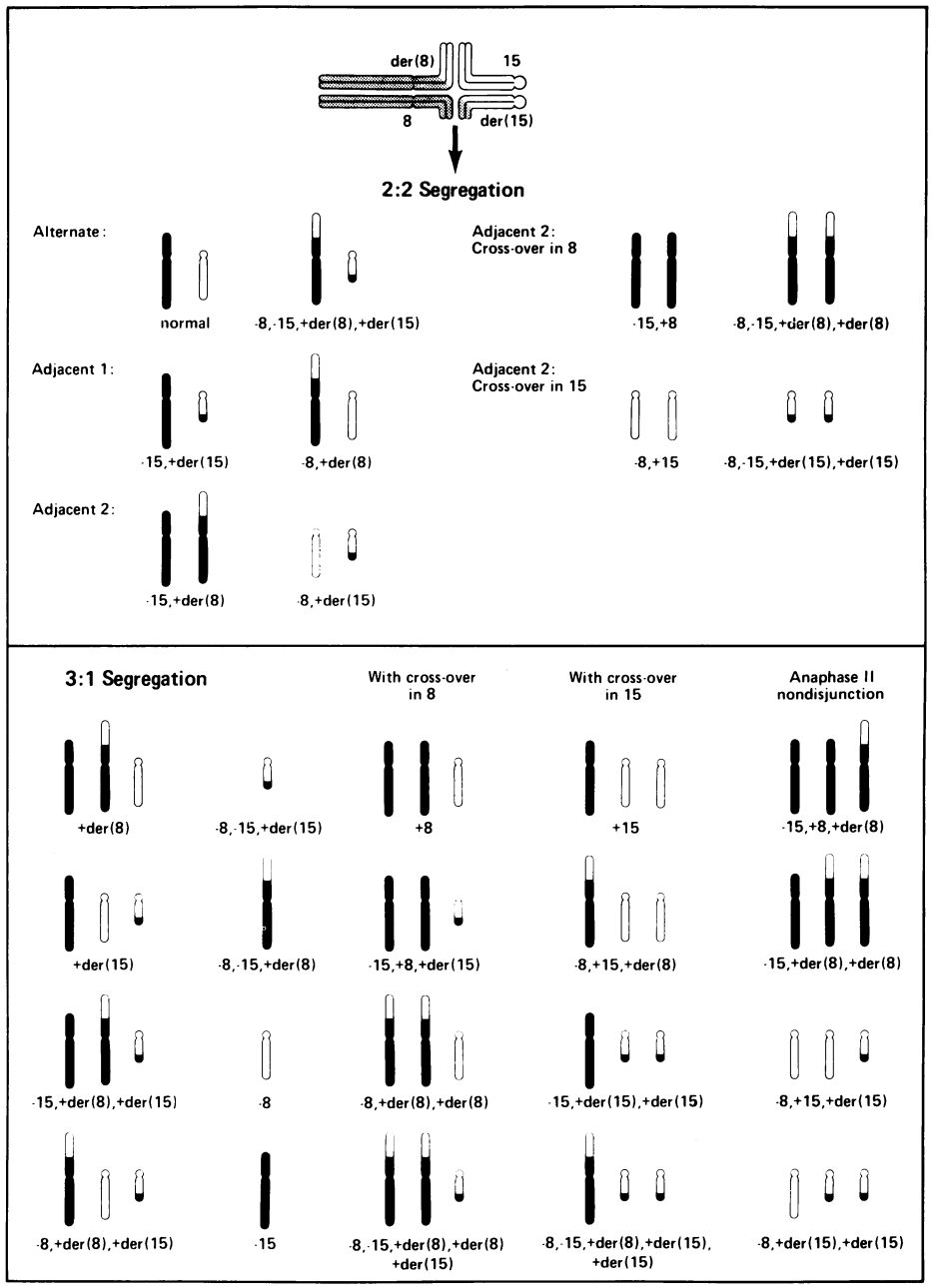

FIG. 1.—Legend appears on next page.

With the introduction of the human-sperm hamster-egg system, in which human sperm chromosomes can be visualized, it has become possible to examine the products of meiotic segregation in mature sperm directly [6–9]. This system allows us to compare actual against predicted segregation ratios. Such comparisons may eventually make it possible to make precise risk calculations for specific translocations, a highly desirable goal in genetic counseling [10]. Here, we report segregation ratios in 427 sperm from two translocation carriers.

MATERIALS AND METHODS

Two translocation heterozygotes were contacted through the genetic counseling services of the University of California Medical Center, San Francisco.

The first donor (TA), 31 years of age, has the karyotype 46,XY,t(8;15)(p22;q21). His medical history indicates hypercholesterolemia and hypertriglyceridemia with secondary pancreatitis. The reciprocal translocation was ascertained during investigation following the birth of a daughter with multiple congenital abnormalities who died at 4 days of age. The deceased child carried an unbalanced form of the translocation; her karyotype was 46,XX, −8, +der(8). This birth was preceded by two spontaneous abortions at 14 weeks gestation (no chromosomal analyses performed) and followed by the birth of a son who also carries the balanced form of the translocation, as well as the birth of another daughter with a normal 46,XX karyotype. Chromosome analysis of the donor's mother also indicated a normal 46,XX karyotype.

The second donor (TC), 25 years of age, has the karyotype 46,XY,t(3;16)(p23;q24). The mother of the donor was first ascertained to be a balanced carrier when she underwent amniocentesis indicated for advanced maternal age. The fetus from that pregnancy was heterozygous for this translocation and for an apparent de novo translocation between chromosomes 1 and 6. Subsequently, all four of her children were tested, and one son, donor TC, was also found to be heterozygous for the (3;16) translocation. The mother had two previous spontaneous abortions at 10 and 12 weeks. The family history is otherwise negative for mental retardation and/or congenital abnormalities.

Donor TA provided one semen sample; chromosome complements were obtained

FIG. 1.—(*Top*), Pachytene diagram and 2:2 segregation illustrated for t(8;15)(p22;q21). Meiotic segregation of a reciprocal translocation results in gametes carrying various combinations of the two translocation chromosomes and their two normal homologs. *2:2 segregation* (two of these four chromosomes pass to each daughter cell) occurs in three ways. Gametes arising from *alternate* segregation have either the two normal homologs or the two translocation chromosomes, resulting in cells with balanced chromosomal consititution. In *adjacent 1* segregation, the homologous centromeres pass to opposite poles, whereas in *adjacent 2*, they pass to the same pole; adjacent segregation results in chromosomal constitutions with various deficiencies and duplications. If crossing over takes place in the interstitial segments (the section between the centromere and the translocation breakpoint) and is followed by alternate or adjacent 1 segregation, the results cannot be cytologically distinguished from noncrossover events. However, when adjacent 2 segregation subsequent to interstitial crossover occurs, some of the combinations that arise are unique and the chromosome involved in the crossover can be identified. These cells show complete hyperploidy of one of the four involved chromosomes [1]. (*Bottom*), 3:1 segregation illustrated for t(8;15)(22;21). Occurring less frequently, the translocated chromosomes and their homologs can disjoin by *3:1 segregation* (three chromosomes to one daughter cell and one chromosome to the other) or even *4:0 segregation* (all four chromosomes pass to one of the daughter cells). Crossing over in the interstitial segments followed by 3:1 segregation can result in unique combinations of chromosomes that can be distinguished cytologically, although nondisjunction at anaphase II can also explain these configurations. Theoretically, several other combinations could arise that can be explained only by nondisjunction during anaphase II [2].

from sperm stored 1 day and 3 days in TEST yolk buffer at 4°C [11]. Donor TC contributed two semen samples, and chromosomes were obtained after 1-day storage in TEST yolk buffer. After 3-day storage, sperm from donor TC did not penetrate eggs. Gamete coincubation, egg culture and fixation, and chromosome analysis were as described [12, 13].

RESULTS

Donor TA, t(8;15)(p22;q21)

The relative proportions of each segregation type from 226 chromosome spreads analyzed for this donor are presented in table 1. We observed 43 normal and 41 balanced complements, 86 adjacent 1 and 48 adjacent 2 karyotypes, as well as eight complements derived by 3:1 segregation. Whereas normal and balanced complements occurred in equal proportions, the frequencies of the two types of adjacent 1 differed from each other ($P < .02$, χ^2 test for dependent proportions). The proportion of unbalanced complements was 142/226 or 62.8%, with 8/226 or 3.5% arising by 3:1 segregation.

Eighteen adjacent 2 and 3:1 complements were observed that could be attrib-

TABLE 1

SEGREGATION OF SPERM CHROMOSOMES FOR A DONOR HETEROZYGOUS FOR RECIPROCAL TRANSLOCATION t(8;15)(p22;q21)

Segregation mode	Sperm chromosome complements*	No. spreads	%
Alternate	23,X or Y	43	19.0
	23,X or Y, −8, −15, +der(8), +der(15)	41	18.1
		84	37.2
Adjacent 1	23,X or Y, −8, +der(8)	54	23.9
	23,X or Y, −15, +der(15)	32	14.2
		86	38.1
Adjacent 2	23,X or Y, −8, +der(15)	11	4.9
	23,X or Y, −15, +der(8)	21	9.3
	23,X or Y, −8, +15†	10	4.4
	23,X or Y, −8, −15, +der(15), +der(15)†	4	1.8
	23,X or Y, −15, +8‡	2	0.9
		48	21.2
3:1	24,Y, +der(8)	1	0.4
	24,X, +der(15)	1	0.4
	24,Y, +15†	1	0.4
	24,X, −8+,der(8), +15†	1	0.4
	22,X or Y, −8	2	0.9
	22,X, −15	1	0.4
	22,Y, −8, −15, +der(15)	1	0.4
		8	3.5

* Aneuploid events and structural aberrations unrelated to the translocation were disregarded when assigning karyotypes to these categories.

† Crossing over within the interstitial segments of 15 or nondisjunction at anaphase II.

‡ Crossing over within the interstitial segments of 8 or nondisjunction at anaphase II.

uted to crossing over in the interstitial segments of the translocation chromosomes (table 1), although nondisjunction at anaphase II could also account for these configurations in the 3:1 spreads [2]. Apparent crossovers in chromosome 15 (16 events) were significantly elevated over those in chromosome 8 (two events; $P < .01$, χ^2 test for dependent proportions, based on relative lengths of interstitial segments [14]).

Figure 2 shows a balanced karyotype for this translocation, figure 3 shows examples of adjacent 1 and adjacent 2 segregation types, and figure 4 shows an example of 3:1 segregation following a probable crossover event.

The frequencies of abnormalities unrelated to the translocation were 2.7% for structural aberrations, 1.3% for hyperhaploidy, and 1.3% for hypohaploidy. The abnormal complements are detailed in table 2. The frequency ratio of X- and Y-bearing sperm was 45.1% and 54.9%, respectively, which did not differ significantly from the expected 50%.

Donor TC, t(3;16)(p23;q24)

For this donor, 201 chromosome spreads were analyzed. Table 3 shows the relative proportions of each segregation type. We observed 40 normal, 35 balanced, 83 adjacent 1, and 33 adjacent 2 complements, as well as 10 derived by 3:1 segregation. In this translocation, the frequencies of the two adjacent 2

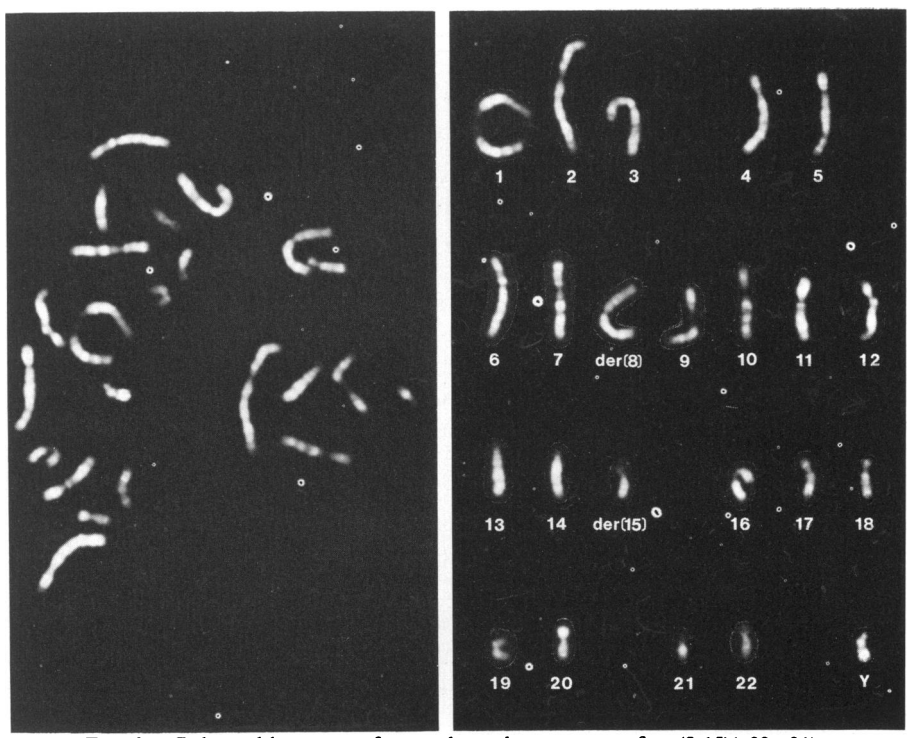

FIG. 2.—Balanced karyotype from a donor heterozygous for t(8;15)(p22;q21)

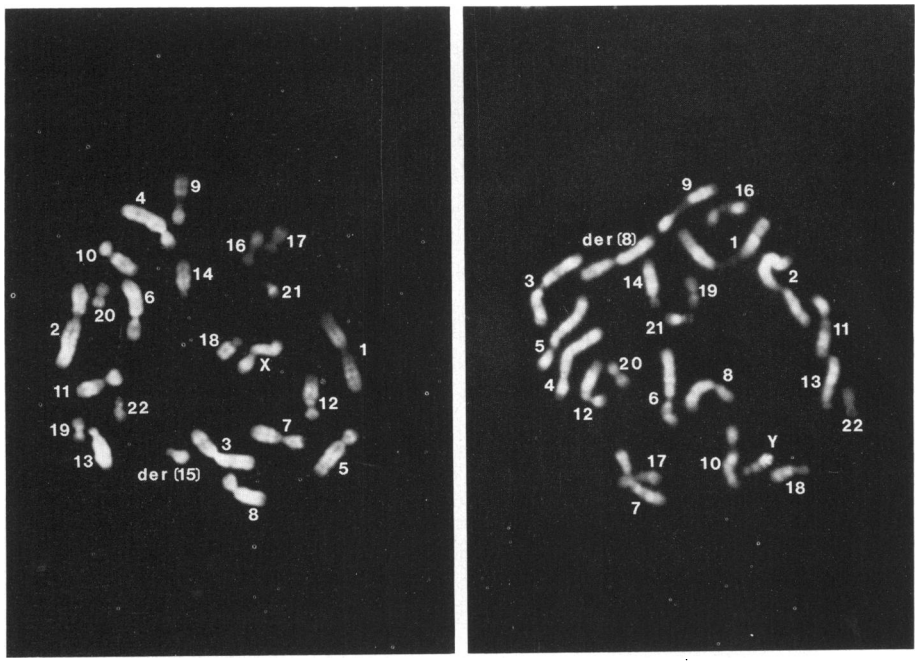

FIG. 3.—(*Left*), Sperm chromosome complement showing adjacent 1 segregation: 23,X, − 15, +der(15). (*Right*), Sperm chromosome complement showing adjacent 2 segregation: 23,Y, − 15, +der(8).

segregation types derived without crossover were different ($P < .05$). The proportion of unbalanced complements was 126/201 or 62.7%, and the 3:1 segregation frequency produced by the translocation, 10/201 or 5%. Four adjacent 2 and two 3:1 complements were observed that could be attributed to crossing-over events in the interstitial segments of chromosomes 3 and 16. Figure 5 shows a balanced karyotype for this translocation.

Abnormalities not associated with the translocation were 5.6% for structural aberrations, 0.5% for hyperhaploidy, and 1.0% for hypohaploidy (table 2). The percentage of X- and Y-bearing sperm was 54.5% and 45.5%, respectively.

DISCUSSION

The two translocations shared several characteristics. They each produced equal numbers of normal and balanced complements from alternate segregations, and the combined numbers of alternate segregation products were not different from the number of adjacent 1 complements. In both translocations, 63% of chromosome spreads were unbalanced, and 3:1 disjunction was low. The frequencies of hyper- and hypohaploidy not related to the translocations were similar to those in 11 normal men, and the frequencies of structural aberrations unrelated to the translocations fell in the lower end of the range for the same 11 normal donors [13].

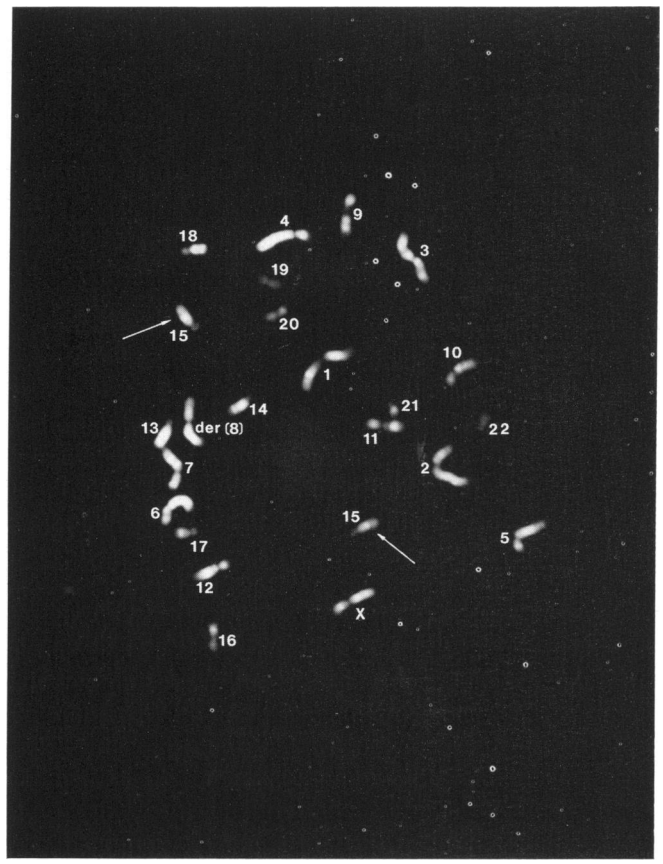

FIG. 4.—Sperm chromosome complement showing 3:1 segregation following crossover in the interstitial segment of 15: 24,Y, −8, +der(8), +15.

The proportions of unbalanced chromosome spreads were considerably higher than the 18.8% and 31.6% reported in 32 sperm complements analyzed for t(5;18) and 19 complements analyzed for t(6;14), respectively [8]. On the other hand, an 11;22 translocation with 77% unbalanced complements among a total of 13 analyzed [7] suggests an even higher frequency than the present results. In both of our translocation carriers, alternate and adjacent 1 frequencies were the same. Balkan and Martin [8], on the other hand, reported alternate frequencies greater than those of adjacent segregation. These differences seem to affirm previous observations that ratios of segregation products vary depending on the translocations involved.

For both of the translocations described here, some sperm complements resulting from adjacent 2 or 3:1 segregation were seen for which the most likely explanation seems to be crossing-over events in the interstitial segments, although nondisjunction at anaphase II cannot be ruled out (tables 1 and 3). In the 8;15 translocation, there appeared to be a bias for crossovers involving

TABLE 2

ABNORMAL SPERM CHROMOSOME COMPLEMENTS FROM TWO TRANSLOCATION CARRIERS

Donor	Abnormal sperm chromosome complement
TA*	23,X, − 8, + der(8),csb(6cen)
	23,X, − 8, − 15, + der(8), + der(15),csb(12p12)
	23,Y, − 8, − 15, + der(8), + der(15),csb(17q21)
	23,Y, − 8, − 15, + der(8), + der(15),del(6)(p21-22→pter)
	23,X, − 8, − 15, + der(8),der(15),del(9)(q1→qter)
	23,Y, − 8, + der(8),del(9)(q11-13→qter)
	24,X, − 15, + 8, + 1
	24,Y, − 8, − 15, + der(8), + der(15), + 21
	24,X,Y
	21,Y, − 8, + 15, − 5, − 9
	22,Y, − 8, + der(8), − 17
	22,Y, − 8, + der(8), − 18
TC	23,Y, − 16, + der(16),dic(1;14)(cen;q3), + ace
	23,Y,inv(10)(p11q22)†
	23,Y, − 3, − 16, + der(3), + der(16),inv(10)(p11q22)†
	23,X, − 3, − 16, + der(3), + der(16cen),csb(1cen)
	23,Y,csb(3q21)
	23,Y,csb,(9p1)
	23,X, − 3, + der(16),csb(11cen)
	23,X, − 16, + der(16),csb(17q21)
	23,X, + ace
	23,X,del(7)(p11-12→pter)
	23,X, − 3, + der(3),del(10)(p11-12→pter)
	27,X, − 16, + der(3), + der(16), + 1, + 2, + 10
	22,0, − 3, + der(3), − X or Y
	22,0, − 3, + der(3), − X or Y

* A human complement containing 28 chromosomes (+ 1, + 7, + der(15), + ?, + ?) was also seen, but was not included since not all the chromosomes involved could be identified.

† Both spreads containing this inversion were recovered from the same egg.

chromosome 15, but no bias was evident in the 3;16 translocation. Of the four chromosomes involved in the two translocations, chromosomes 3 and 16 had the largest interstitial segments followed by chromosome 15 and by chromosome 8 with the shortest [14]. Theoretically, relative numbers of recombination events should have occurred in the same order (i.e., $3 > 16 > 15 > 8$). Instead, cytogenetically identifiable crossovers involving chromosomes 3, 8, and 16 each accounted for 1% of the total number of complements analyzed per donor, whereas chromosome 15 crossovers were evident in 6% of the spreads from donor TA. This suggests that factor(s) other than interstitial segment length may be contributing to recombination.

In each of the two donors, one cell was observed with several hyperploid events. TC had one cell with 27 (table 2) and TA had one cell with 28 chromosomes (see footnote to table 2). Both complements were derived probably by 3:1 disjunction, so that in each cell at least one of the extra chromosomes could be accounted for by the translocation. However, among 2,468 karyotypes obtained from 11 normal men, there was not a single cell with greater than 24

TABLE 3

SEGREGATION OF SPERM CHROMOSOMES FOR A DONOR HETEROZYGOUS FOR RECIPROCAL TRANSLOCATION t(3;16)(p23;q24)

Segregation mode	Sperm chromosome complements*	No. spreads	%
Alternate	23,X or Y	40	19.9
	23,X or Y, −3, −16, +der(3), +der(16)	35	17.4
		75	37.3
Adjacent 1	23,X or Y, −3, +der(3)	43	21.4
	23,X or Y, −16, +der(16)	40	19.9
		83	41.3
Adjacent 2	23,X or Y, −3, +der(16)	20	10.0
	23,X or Y, −16, +der(3)	9	4.5
	23,Y, −3, +16†	1	0.5
	23,Y, −3, −16, +der(16), +der(16)†	1	0.5
	23,X, −16, +3‡	2	1.0
		33	16.4
3:1	24,X or Y, +der(3)	2	1.0
	24,X or Y, −16, +der(3), +der(16)	2	1.0
	24,X or Y, −3, +der(3), +der(3)‡	2	1.0
	22,X or Y, −3	2	1.0
	22,Y, −3, −16, +der(3)	1	0.5
	22,Y, −3, −16, +der(16)	1	0.5
		10	5.0

* Aneuploid events and structural aberrations unrelated to the translocation were disregarded when assigning karyotypes to these categories.

† Crossing over within the interstitial segments of 16 or nondisjunction at anaphase II.

‡ Crossing over within the interstitial segments of 3 or nondisjunction at anaphase II.

chromosomes [13]. Reports of spontaneous abortuses with greater than single trisomic constitution are quite rare, although one abortus with a triple trisomy has been reported [15]. On the other hand, the association of reciprocal translocations with unrelated trisomies has been noted by several authors [8, 16–18]. Interchromosomal effects (reviewed by Lima-de-Faria [19]) have been invoked to account for these associations: in particular, distributive pairing at meiosis [20]. Familial unrelated balanced translocations may represent another cytogenetic category and mechanism for Down syndrome [21]. In fact, increased nondisjunction in uninvolved bivalents has been shown to occur in female, although not in male, translocation-carrying mice [22].

The overall frequencies of 3:1 segregation products were 3.5% in the t(8;15) and 5% in the t(3;16) heterozygote. These events were evenly divided between nullisomy and disomy, as expected on theoretical grounds. The frequency of 3:1 products involving only the normal (and not the derivative) chromosomes induced by 3:1 disjunction ranged from 1% to 2% (tables 1 and 3) and can be considered to be an inherent, or background, property of these translocations. In programs testing for environmentally induced chromosomal abnormalities,

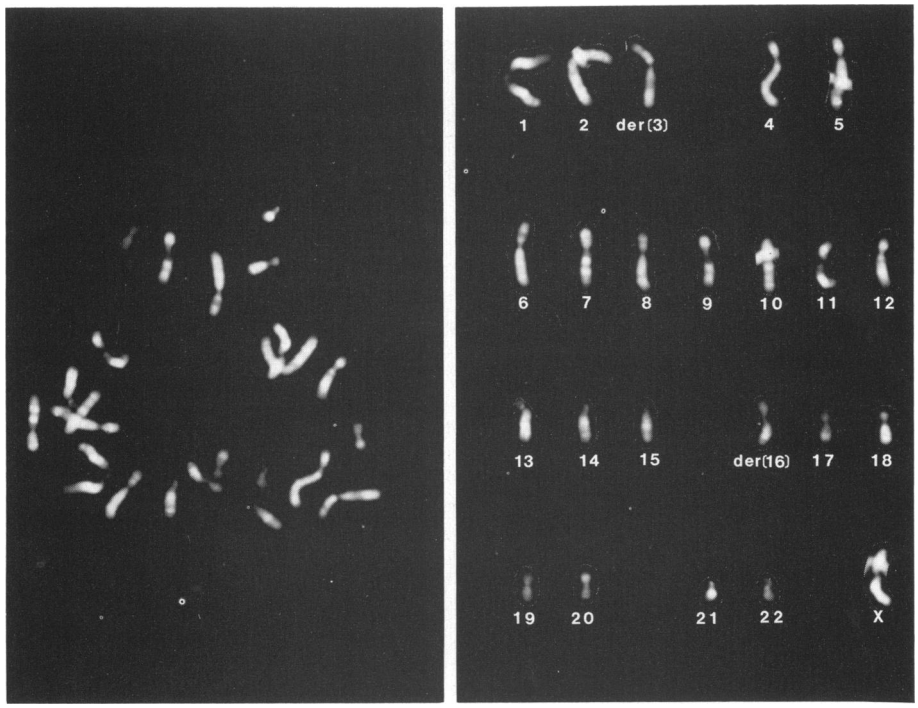

FIG. 5.—Balanced karyotype from a donor heterozygous for t(3;16)(p23;q24)

3:1 complements involving only the normal chromosomes would be indistinguishable from aneuploid events unrelated to translocations. This background apparent aneuploidy due to translocation segregation would have to be subtracted to estimate more accurately the true frequency of induced aneuploidy.

In t(3;16), two complete, separate chromosome complements were observed, each with a pericentric inversion of chromosome 10 involving identical breakpoints (table 2). Both of these complements were recovered from a single egg. Since removal of the zona pellucida (see MATERIALS AND METHODS) destroys the block to polyspermy in these eggs, we cannot say with certainty whether these two complements were derived from two separate sperm or from a double-headed sperm. However, it can be argued that these complements, in fact, came from a double-headed sperm, because in our previous analysis of 2,468 karyotypes [13], we never observed an inversion. In addition, both of these complements contained a Y chromosome and the translocation chromosomes disjoined by alternate segregation in each complement, one containing both normal chromosomes, the other both derivative chromosomes. This particular chromosomal constitution would have had to arise from an anaphase II event, most likely an incomplete cytokinesis.

The methods and data described herein should prove to be important adjuncts to genetic counseling. Frequencies of likely reproductive outcomes at present are calculated largely on the basis of empirical data from sibships [4].

Direct inspection of sperm chromosomal complements in translocation carriers should make it possible in the future to add to the precision of these predictions.

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GENETICS IN FICTION: CONGENITAL APLASIA OF THE NIPPLE. Upon occasion, genetic matters occupy a place in fiction. To illustrate this, let us examine a contemporary novel, *The Prodigal Daughter* (New York: Linden Press/Simon & Schuster, Inc., 1982) by Jeffrey Archer. Archer was born in 1940, became the youngest member of the House of Commons in 1969, and served there until 1974. He is a novelist and lives in Cambridge. In *The Prodigal Daughter,* the heroine, Florentyna Rosnovski, is told as a child by her father Baron Abel Rosnovski of his noble origin:

> As the days went by, Abel revealed to his daughter how his sister Florentyna, after whom she has been named, joined him in the castle and the way he discovered the Baron was his real father.
>
> "I know, I know how you found out," cried Florentyna.
>
> "How can you know, little one?"
>
> "He only had one nipple," said Florentyna. "It must be, it must be. I've seen you in the bath. You only have one nipple, so you had to be his son. All the boys at school have two. . . ." Abel and Miss Tredgold stared at the girl in disbelief as she continued, "but if I'm your daughter, why have I got two?"
>
> "Because it only passed from father to son. . . ."

At this point it would appear that the development of only one nipple is a Y-linked trait. But Abel continues by telling Florentyna that, as regards aplasia of the nipple:

> "[It] is almost unknown in daughters."
>
> "It's not fair. I want only one."
>
> Abel began laughing, "Well, perhaps if you have a son, he'll have only one."

One hundred or so pages later in the narrative, Florentyna has grown-up, married, and just had a baby:

> ". . . a large son of nine pounds three ounces. He had only one nipple."

The transmission genetics are clear. Congenital aplasia of the nipple is an X-linked trait usually with expression largely male limited. That is how this genetic matter stands, in fiction. (*Frederick Hecht*)