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Analysis of Meiotic Segregation in a Man Heterozygous for Two Reciprocal Translocations Using the Hamster in Vitro Penetration System

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SUMMARY

Sperm chromosomal complements of a man heterozygous for two reciprocal translocations and exhibiting the karyotype 46,XY,t(5;11)(p13;q23.2),t(7;14)(q11;q24.1) were analyzed following in vitro fusion with golden hamster zona-free eggs (the hamster in vitro penetration [HIP] system). Products of alternate, adjacent 1, and 3:1 segregation at meiosis I of both translocation quadrivalents were recovered, and the analysis of their output, which was dissimilar between the two translocations, permitted prediction of probable sites of chiasma formation in the chromosomes involved in the translocation. These data, which comprise the first reported analysis of the products of two translocations in a single individual (hence, in a common genetic background), emphasize the uniqueness in genetic behavior of individual translocations; they further demonstrate the usefulness of the HIP system to carry out such studies.

INTRODUCTION

Balanced reciprocal translocations occur in humans at an approximate frequency of 1/550 live births [1]. They are important clinically because conceptions of carrier individuals are at risk for inheriting unbalanced chromosomal complements that are predisposed to spontaneous abortion or, in the event of

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survival to term, for developmental defects. The genetics of translocations has been well investigated in a variety of non-human species (plants, insects, rodents) [2] because, in these species, spermatocyte and oocyte materials usually are available for direct cytological analysis of rearranged chromosomes at meiosis. In addition, in these species, the chromosomal complements of products of fertilization can be investigated systematically in appropriate cross-breeding experiments. In contrast, data on the genetics of human translocations have been difficult to obtain because of their general rarity and the many problems associated with obtaining gametic and zygotic material for cytogenetic analysis. The data available mostly are anecdotal karyotype analyses of rearrangement carriers and their progenies referred to clinical cytogenetics laboratories for evaluation of recurrent fetal wastage or birth of a developmentally abnormal child [3–5].

Zona-free golden hamster eggs support penetration and activation of capacitated human sperm in vitro [6]. The frequency of activation of sperm from a given semen sample is expressed as the proportion of eggs with activated or enlarged sperm heads. Termed as sperm penetration assay (SPA), this parameter correlates roughly with the fertility status of the sperm donor (fertile men exhibit SPA values of 10% or more) [7]. When chromosome condensation in the penetrated eggs is allowed to take place in the presence of Colcemid, suitably spread metaphase plates can be obtained and the karyotypes of the hamster and human chromosome complements can be analyzed after appropriate staining (Q-banding in this case) to reveal banding patterns [8]. The availability of this system has two major advantages for the genetic analysis of translocations: (1) it enables analysis of the meiotic consequences of rearrangements in the male far more rigorously than had been possible until now [9, 10] due to the fact that there is no selection against unbalanced zygotes, and (2) it is noninvasive for the subject, requiring only a semen sample. In contrast, study of meiosis in spermatocytes requires a testicular biopsy. We have studied, using the hamster in vitro penetration (HIP) system, the cytogenetics of sperm from an unusual individual who carried two balanced reciprocal translocations involving chromosomes 5 and 11 in one translocation and 7 and 14 in the other. Based on the analysis of the sperm chromosomal complements recovered, we have reconstructed the probable meiotic behavior of the two sets of translocations in this man.

MATERIALS AND METHODS

Ascertainment of the Family

The family (fig. 1) was referred to us for prenatal cytogenetic evaluation of the wife's pregnancy because she had previously given birth to a child with the cri-du-chat syndrome [chromosomal complement: 46,XX,t(7;14)(q11.23;q24.1),del(5)(p13→ter)]; in addition, she also had four first-trimester spontaneous abortions. The amniotic fluid cells from this pregnancy displayed the chromosomal complement 46,XY,t(7;14)(q11.23;q24.1), and a normally developed male infant born at term also had the same chromosomal complement. The proband's chromosomal constitution was determined to be 46,XY,t(5;11)(p13;q23.2),t(7;14)(q11.23;q24.1) (fig. 2).

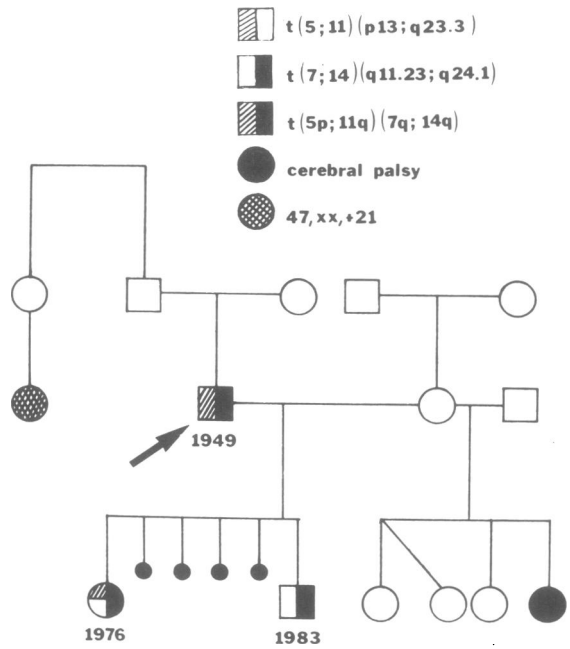

FIG. 1.—Pedigree of the family in which the two translocations are segregating

Culture Media and Their Preparation

Three types of culture media prepared according to the method described by Rudak et al. [11], namely, (1) Biggers-Whitten-Whittingham (BWW) medium, (2) BWW supplemented with 15 mg/ml albumin, and (3) Ham's F-10 medium (Gibco, Grand Island, N.Y.) supplemented with 15% heat-inactivated fetal bovine serum (FBS) (Gibco) were used in the HIP experiments. The BWW media were prepared prior to each experiment. The pH and temperature of the medium were found to be critical factors in the success of the experiment. The medium was adjusted to pH 7.4 just prior to sterile filtration and was kept at 37°C. Oil was not used to cover the medium at any time during the experiment.

Superovulation of Golden Hamsters

The animals (Charles River, Mass.) were kept on a timed light cycle of equal hours of light and darkness. On day 1 of their estrus cycle (determined by vaginal discharge), three females were routinely injected with 25 I.U. of pregnant mare's serum (PMS) (Gestyl, Organon). On day 3, 16 hrs prior to sacrifice, the animals were injected with 24 I.U. of human chorionic gonadotropin (hCG) (Sigma, St. Louis, Mo.).

Processing of Semen Samples

The fresh ejaculate was kept at room temperature in a sterile air-tight container (Falcon 4013) for 20 min. After liquification, 0.1 ml of it was diluted in 0.9 ml of spermacide [7] and used for a sperm count on a hemocytometer. The remaining semen was diluted with an equal volume of BWW and filtered through two layers of sterile kimwipes. It was washed in BWW by centrifugation at 600 *g*. This procedure was repeated twice, and the resulting pellet was resuspended in the albumin-enriched BWW. Before being aliquoted into sterile Falcon tubes (#2003), the sperm count and motility

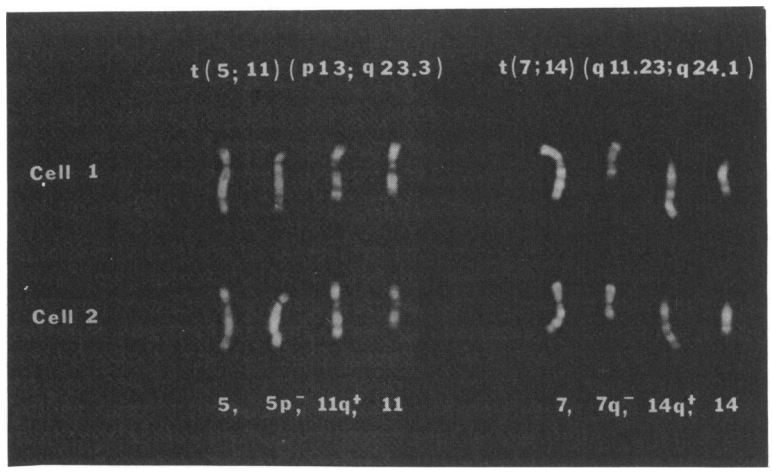

FIG. 2.—Quinacrine-banded partial karyotypes from two phytohemagglutinin-stimulated blood lymphocytes from the proband showing the two reciprocal translocations, t(5;11)(p13;q23.2) and t(7;14)(q11.23;q24.1).

were ascertained and the former adjusted such that each tube held 10×10^6 sperm/ml medium. The tubes, with loosened caps, were placed in a CO (3%) incubator for in vitro capacitation, the duration of which varied from 4 to 40 hrs.

SPA

The oocytes and sperm were mixed together in 1 ml of BWW at a final sperm concentration of 1×10^6. After 3 hrs incubation, 20 oocytes were removed and evaluated for sperm penetration. This was carried out by placing them on alcohol-cleaned glass slides that had previously been prepared with four vaseline-paraffin posts (16:1). The purpose of these posts was to provide sufficient room between the slide and the coverslip for the mounting medium (BWW) in order to prevent drying of the oocytes during microscopy as well as keeping them from being crushed by the weight of the coverslip. The oocytes were placed in the center of the posts, and a 22 × 30-mm glass coverslip (Corning) was gently placed over the posts and pressed down until the oocytes began to flatten slightly. They were examined at this stage under direct phase optics (400×) for presence of expanded sperm heads. Following this, the oocytes were fixed with 3:1 methanol-acetic acid, which was accomplished by placing a drop or two of the fixative on one edge of the coverslip and drawing off the BWW medium at the opposite side with a piece of filter paper. Fixed oocytes were stained with 1% aceticlacmoid following the same procedure as with fixation.

Oocyte Chromosome Preparation

Oocytes destined for chromosome analysis were placed in Ham's F-10 medium and incubated overnight after being mixed with sperm. Colcemid (0.5 mg/ml) was added 7–15 hrs after mixing the gametes. The next morning, the eggs were placed in 1% sodium citrate for 5 min and then transferred to the center of precleaned slides. The cells were fixed in the following manner. As much fluid as possible surrounding the oocyte was removed, and a small drop of 3:1 methanol-acetic acid fixative was placed on the slide half-way between the cells and the edge of the glass. The resulting wave of fixative then moved gently over the eggs without disturbing their positions. Once the first wave of fixative passed over the cells, a second and then a third drop were placed on the slide but

closer to the cells than the first drop, making sure that as each drop was added the preceeding one had almost evaporated. During fixation, the oocytes first rounded up, then expanded, and finally disappeared from view. The entire hyposmosis-fixation procedure was carried out while observing the oocytes through a stereobinocular microscope (20×). Just prior to the drying out of the fixative, the slide was picked up from the microscope stage and gently blown upon to aid in the spreading of the chromosomes. Aging and drying the slides for 2 weeks in a closed slide box prior to staining helped to bring out the banding patterns. Q-banding was routinely used [12].

RESULTS

Fresh semen samples were obtained from the proband on four separate occasions and penetration experiments were set up for SPA evaluation and chromosome analysis as described above. The proband's sperm counts ranged from 50 to 140 × 10^6/ml, and his mean SPA was 48% (range: 26%–70%); both were within the limits of values for normal fertile men published in the literature [7, 13] as well as those observed in our laboratory.

The penetration experiments yielded 23 sperm (seven from 4 hrs capacitation, seven from 6 hrs capacitation, and nine from 40 hrs capacitation) whose karyotypes were informative for both sets of translocated chromosomes (a representative sperm karyotype is illustrated in fig. 3). These data, summarized in table 1, clearly show that although both translocations underwent alternate, adjacent 1, and 3:1 modes of segregation during meiosis I the frequencies of these disjunctions differed between the two translocations. Thus, only four of the 23 (13.73%) cells represented products of alternate segregation of both translocations. In the case of t(5;11), products of alternate segregation were significantly more frequent than those of adjacent I segregation (16 vs. six, $P < .01$) and, among the products of alternate segregation, normal karyotypes were significantly in excess of balanced ones (14 vs. two, $P < .001$). In the case of t(7;14), the difference between the products of alternate and adjacent modes of segregation was insignificant (seven vs. 11, $P > .2$); however, products of nondisjunctional segregation (3:1 and 4:0) comprised 20% of cells in comparison to 4% in the case of the previous translocation. In addition, second meiotic nondisjunction of chromosome 14 was observed in three of 23 cells (13%), once each in the products of alternate, adjacent 1, and 3:1 segregations. We have noted additional chromosome abnormalities in the 23 sperm analyzed in the HIP system. Of the 23 cells, nine were nullisomic haploids (n − 1) and seven were disomic haploids (n + 1), losses and gains of chromosomes in them was random; the remaining seven were complete haploids. Chromosome breaks were encountered in three cells. Among the 23 sperm, 17 were Y-bearing and five X-bearing, and the remaining one had both an X and a Y chromosome. In summary, 69.5% of the sperm karyotypes examined had chromosome abnormalities in addition to the unbalanced products of the translocations; not a single normal complement was encountered in the cells studied. In contrast, 26 sperm complements analyzed from two normal individuals (14 metaphases and 12 metaphases, respectively) yielded 2 (7.7%) abnormal karyotypes. Both abnormal sperm were from the same individual (the man with 14 karyotypes

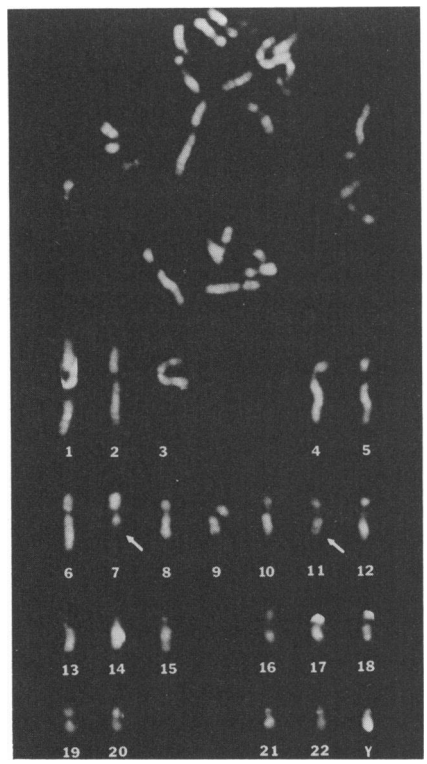

FIG. 3.—Quinacrine-banded chromosome spread (and its karyotypic display) from a sperm from the proband that underwent activation in the HIP system. Both translocations must have undergone adjacent 1 segregation to give rise to this cell that is unbalanced for chromosomes 5, 7, 11, and 14 [chromosomes 7 and 11 are replaced by der(7) and der(11)].

analyzed); one was missing a no. 21 chromosome and the other had an extra no. 21 chromosome.

DISCUSSION

In order to deduce the basis for the yield of gametic chromosomal complements observed, we reconstructed the most likely meiotic behavior (pachytene pairing, diakinesis chiasmata, metaphase orientation, and anaphase segregation) of the two translocations (fig. 4) taking published data on spermatocyte chiasma patterns into consideration [14]. In the case of t(5;11), the products of concordant segregation (equal segregation of centromeres at anaphase I leading to alternate and adjacent 1 orientation) of a ring quadrivalent following chiasma formation in the centric (chiasma positions A, F, and G in fig. 4*A*) and noncentric (chiasma positions C and D in fig. 4*A*) segments would give rise to the observed structurally balanced and unbalanced complements while discordant segregation of the ring (unequal segregation of centromeres at anaphase 1) would give rise to the single nondisjunctional gamete observed. Alternatively,

TABLE 1

POSTULATED ORIGIN OF SPERM COMPLEMENTS OBSERVED BASED ON INDEPENDENT AND JOINT SEGREGATION OF THE TWO TRANSLOCATIONS

Germ-line translocation	Complement in sperm	No. sperm	Origin by mode of segregation
t(5;11)	Normal 5,11	14	Alternate
	5p−,11q+	2	Alternate
	5p−,11	2	Adjacent 1
	5,11q+	4	Adjacent 1
	5,5p−,11	1	3:1
Total		23	
t(7;14)	Normal 7,14	4	Alternate
	7q−,14q+	3	Alternate
	7q−,14	8	Adjacent 1
	7,14q+	3	Adjacent 1
	7,14,14q+	4	3:1
	−7,−7q−,−14,−14q+	1	4:0
Total		23	
t(5;11);t(7;14)	5,11,7,14	2	
	5,11,7q−,14q+	1	Alternate for both
	5p−,11q+,7,14	1	
	5,11,7q−,14	6	Alternate for t(5;11) &
	5,11,7,14q+	2	Adjacent 1 for t(7;14)
	5p−,11q+,7,14q+	1	
	5,11q+,7q−,14q+	1	Adjacent 1 for t(5;11) &
	5p−,11,7q−,14q+	1	Alternate for t(7;14)
	5,11q+,7q−,14	2	Adjacent 1 for both
Total		17*	

* The remaining six were nondisjunctional gametes (3:1 and 4:0 segregation) listed above.

since 5p is a relatively short segment that normally displays no more than a single chiasma [14], it is likely that chiasma fails to form in this rearranged segment leading to formation of a so-called frying-pan-type configuration during diplotene-diakinesis [15]. (Chiasma suppression in translocated chromosome segments is well known in lower organisms [15]; deviations from chiasma frequencies seen in normals have been reported recently in human translocation carriers [16].) The "frying-pan" configuration generates a chain quadrivalent at metaphase I whose centromeres can coorient concordantly or discordantly, ultimately yielding all the gametic combinations observed (fig. 4*A*). In the case of t(7;14), the most likely meiotic behavior would result in a ring or a chain quadrivalent (fig. 4*B*). Although 14p does not support a chiasma, the proximal region of 14q appears to be the site for a consistent chiasma [14]; therefore, in this translocation, this region becomes an interstitial segment with a chiasma (chiasma position E in fig. 4*B*). If chiasmata are formed in the remaining centric (chiasma position A in fig. 4*B*) and the two noncentric seg-

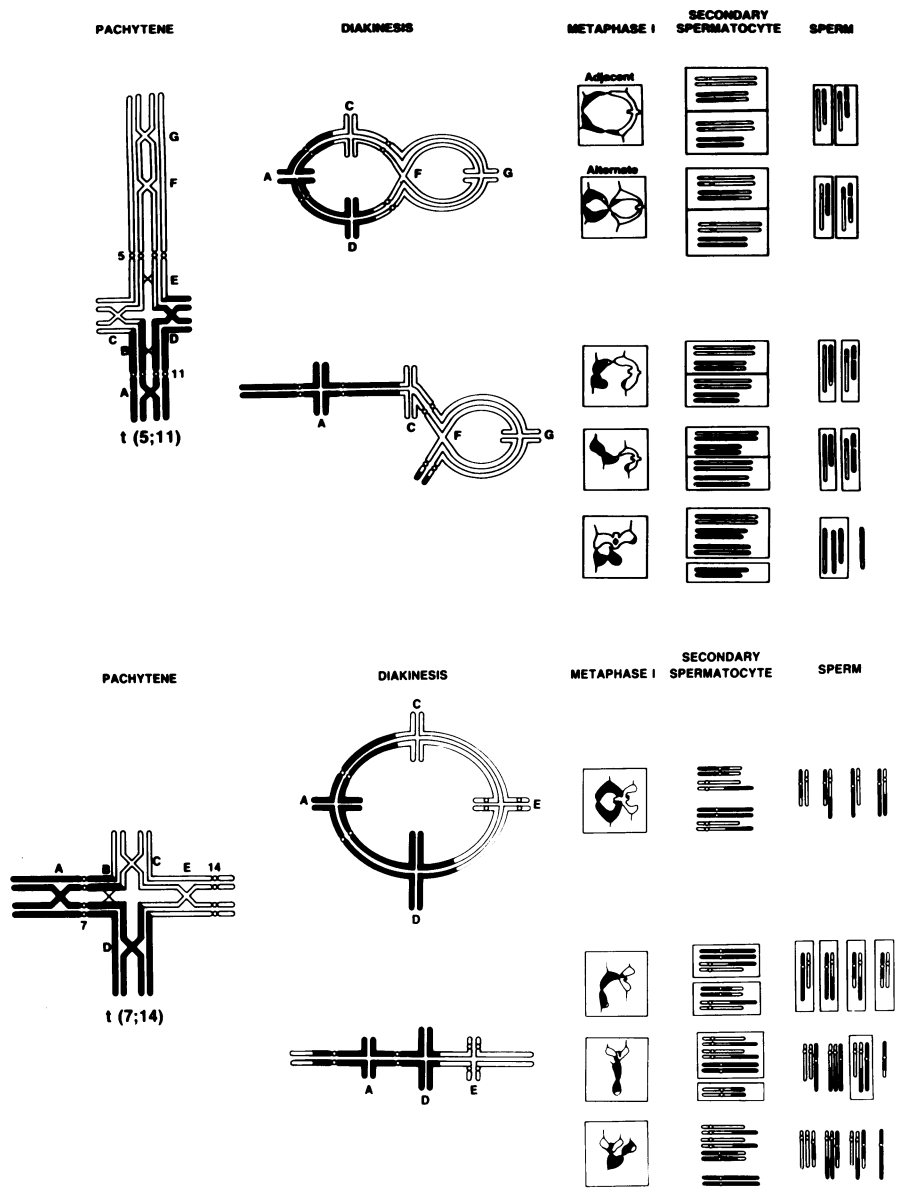

FIG. 4.—Schematic representation of the expected meiotic behavior (pachytene pairing, chiasma positions, diakinesis configurations, metaphase I orientation, and genomes of secondary spermatocytes and sperm) of the two translocations carried by the proband; 4*A* (*top*) refers to t(5;11) and 4*B* (*bottom*) refers to t(7;14). *Letters* indicate positions of chiasma determined on the basis of published chiasma data [14]. Chiasmata in the centric and noncentric segments are represented as exchanges and their cytological consequences have been followed through to the gametes. Chiasmata in the interstitial segments (other than the one in proximal 14q) are represented by an X and their cytological consequences have not been followed through in the illustrations although they will have an effect on the genetic constitution of some of the gametes produced [17]. The gametic genotypes encountered among the proband's sperm that underwent activation in the HIP system are indicated by *boxes* around them.

ments (chiasma positions C and D in fig. 4*B*), then a ring quadrivalent with an interstitial chiasma (chiasma position E in fig. 4*B*) would be generated at diakinesis, which at metaphase 1 will form a "fish-tail" or "branched-ring" quadrivalent (fig. 4*B*). The disjunction of such a structure will invariably be concordant, leading to all numerically balanced complements among which structurally balanced and unbalanced will be in equal proportions. (The terms, alternate and adjacent modes of segregation, become inapplicable in this situation.) However, 16% of the gametes analyzed were the products of nondisjunctional segregation (3:1) of the quadrivalent, thus ruling out the formation of a quadrivalent of the type described above. A probable alternative is failure of chiasma in the rearranged 7q (chiasma position C in fig. 4*B*) which will lead to the formation of a "branched-chain" quadrivalent. The orientation and segregation of such a chain can account for the origin of all the gametic combinations observed, as shown in fig. 4*B*. Failure of chiasmata at positions D and A are unlikely; the region of chiasma D is a long segment comprising approximately 2/3 the long arm of chromosome 7 and should be able to support a chiasma, while failure of chiasma A would result in adjacent 2 segregation, the products of which were not encountered.

The significant difference in the yield of products of alternate and adjacent 1 segregation types of t(5;11) and the difference in the frequency of nondisjunctional segregation between the two translocations reemphasizes the fact that individual translocations tend to be unique in their genetic behavior [2, 17]. Several genetic and nongenetic factors contribute to this behavior. The meiotic orientation behavior of quadrivalents, which determines the anaphase segregation of chromosomes, is dependent on the positions and frequencies of formation of chiasmata in different segments of the pachytene cross [17, 18]. These, in turn, are influenced by the genotype, environment (e.g., temperature), age, and other factors that exercise a control over chiasma formation per se [19, 20]. Furthermore, as discussed above, the occurrence of the rearrangement itself and the size of the translocated segment may act as limiting factors for chiasma formation [15]. In addition, studies of behavior of translocations in plant and insect species have demonstrated that genotypic and environmental factors such as the ones that affect chiasma formation also can exercise control over the types of segregation that translocation quadrivalents undergo during meiosis in the heterozygotes [2]. Human cases such as the present one, although rare (we are aware of only four other reports of individuals heterozygous for two separate reciprocal translocations [21–24]), are of unusual interest because they permit the simultaneous analysis of two sets of translocations that underwent meiosis at the same time in an identical genetic background.

The above data clearly demonstrate the usefulness of the HIP system for the analysis of meiotic behavior of chromosome rearrangements and the gametic output resulting from it in a manner that is noninvasive to the subject. Very few studies of translocation carriers have so far been performed using this system [10, 25–27]. In sperm from carriers of four different translocations, Martin found that the frequency of unbalanced karyotypes due to the translocation ranged from 13% to 77% and other abnormalities in the same cells ranged from

8.3% to 17.9% [27]. While all four translocations generated products of alternate, adjacent 1, and 3:1 segregations, only one, t(11;22) yielded products of adjacent 2 segregation [27]. In sperm from normal individuals, chromosomally abnormal karyotypes have been reported at a frequency of 8.5%–12% [28, 29]. The reason for this excess production of chromosomally abnormal sperm by some translocation carriers is not clear at present. When sufficient data from future studies become available, it would be possible not only to better understand the genetics of human translocations but also to develop estimates of gametic output for translocations involving different chromosomes in a clinically relevant manner.

NOTE ADDED IN PROOF: While this manuscript was in press, Brandriff et al. (*Am J Hum Genet* 38:197–208, 1986) reported on the analysis of sperm karyotypes from two translocation carrier males, t(8;15) and t(3;16), using the HIP system. They observed adjacent 2 segregation in both (21.2% and 16.4%, respectively).

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