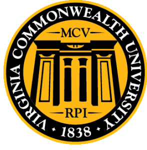

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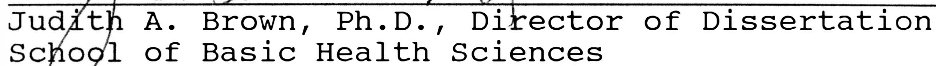

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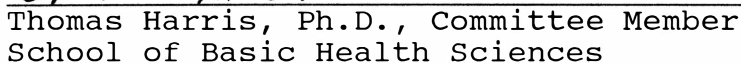

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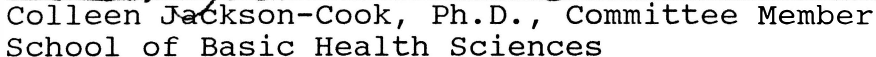

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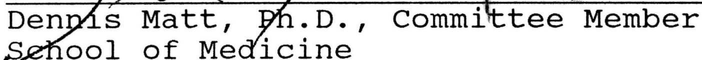

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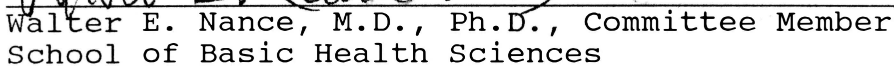

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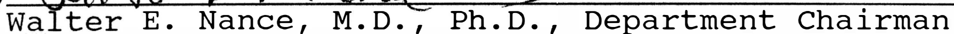

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Molecular and Cytogenetic Characterization of *de novo* Acrocentric Rearrangements in Humans

A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy at Virginia Commonwealth University.

By

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Molecular and Cytogenetic Characterization of de novo Acrocentric Rearrangements in Humans

ABSTRACT

A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy at Virginia Commonwealth University.

Lisa Gail Shaffer

Virginia Commonwealth University

Advisor: Judith A. Brown, Ph.D.

I have studied 26 children who have a de novo rearrangement of the acrocentric chromosomes in order to understand the formation of these aberrations. The families include 25 probands ascertained for Robertsonian-type translocations, 13 between nonhomologous chromosomes and 12 between homologs, and one rea(21;21)(q22;q22). The parental origins of the de novo rearrangements were determined in 26/26 families using QFQ and NOR variants and/or RFLP analyses. While there was no overall difference in the sex distribution of the parents of origin, there were more maternally derived nonhomologous ("true" Robertsonian) translocations (8 mat: 5 pat) and more paternally derived homologous rearrangements (4 mat: 9 pat). A role of the NOR in de novo formation of acrocentric rearrangements was suggested by a significantly

higher incidence of dNOR variants in the parents in whom the rearrangements originated (11/26) as compared to their normal spouses (1/26) and a control population (5/50) ($p<0.0001$). The dNOR variant was found both in parents in whom de novo Robertsonian translocations and homologous rearrangements had occurred. Additionally, both the parents in whom rearrangements originated and their spouses had significantly higher NOR scores than the controls. This suggests that higher NOR scores in the parents may have contributed to the survival of their offspring with de novo acrocentric rearrangements since these rearrangements generally resulted in the loss of two NORs. However, compensation in NOR scores or satellite associations was not evident in these probands. RFLP analysis of rearrangements between homologous chromosomes resulting in secondary trisomy in 8 cases suggested that these rearrangements were isochromosomes, derived from one parental chromosome. Four of the homologous rearrangements were dicentric suggesting that these rearrangements may have resulted from U-type exchanges in the NOR or short arm.

INTRODUCTION

Robertsonian translocations, as first described by Robertson (1916), are whole arm exchanges which take place between acrocentric or telocentric chromosomes. Robertsonian translocation formation is the most common mechanism contributing to karyotypic evolution in plants (Robertson, 1916) and animals (Hsu, 1979). In humans, Robertsonian translocations are the most frequently occurring structural rearrangements and contribute significantly to fetal wastage and malformation/mental retardation syndromes (Jacobs, 1981).

Robertsonian translocations between homologous chromosomes cannot be morphologically distinguished from isochromosomes. Isochromosomes are chromosomes composed of genetically identical arms derived from a single chromosome (Darlington, 1939; 1940). The most common isochromosome in humans is thought to be i(Xq). However, since acrocentric isochromosomes cannot be distinguished from Robertsonian translocations, one cannot exclude the possibility that t(21q21q) are truly i(21q). Rearrangements of chromosome 21 contribute significantly to the occurrence of Down syndrome; the majority are *de novo* rearrangements, rea(21q21q).

One approach to understanding the causes and consequences of Robertsonian-type translocations in humans is to study individuals who have a new or de novo rearrangement and their karyotypically normal parents. The primary objectives of this investigation are; 1) to determine the parental origins of the de novo rearrangements; 2) to identify factors which may influence the formation of new acrocentric rearrangements such as the nucleolar organizer region or satellite associations; and 3) to distinguish between "true" Robertsonian translocations and isochromosomes. Through this investigation it is hoped that factors involved in Robertsonian translocation and isochromosome formation will be identified and increase our knowledge about acrocentric rearrangements.

LITERATURE REVIEW

I. Historical Aspects of Acrocentric Rearrangements

A. Robertsonian Translocations

In 1916, Robertson examined the chromosomes from various species of grasshoppers (Robertson,1916) and concluded that the V-shaped chromosomes seen in spermatocytes were two non-homologous chromosomes permanently associated at the primary constriction (centromere). The appearance of V-shaped chromosomes in some species accounted for the reduced number of chromosomes seen in certain families of grasshoppers.

The formation of Robertsonian translocations is known to be the most common mechanism in karyotype evolution (Hsu, 1979). Examples of mammals that have been found to carry naturally occurring Robertsonian translocations are given in Table 1. These animals provide an opportunity to observe segregation and fertility in carriers of Robertsonian translocations and allow investigation into the mechanisms of Robertsonian translocation formation, species evolution, and imprinting.

Robertsonian translocations are the most common structural rearrangements in humans (Jacobs, 1981). Polani (1960) first demonstrated a Robertsonian translocation in an

Table 1. Mammals demonstrating Robertsonian translocations and their application as animal models

Species	Application	Source
Chicken	Segregation	Bonaminio and Fechheimer, 1988
Dog	Segregation	Larsen et al., 1979
Sheep	Fertility	Bruere, 1975
		Chapman and Bruere, 1975
		Long, 1977
		Long, 1978
		Bruere et al., 1981
Mouse	Evolution	Capanna et al., 1975, 1976
	Mechanisms	Miller et al., 1978
	Segregation	Ruvinsky et al., 1987
	Fertility	Redi et al., 1985, 1988
	Imprinting	Cattanach, 1986
Cow/ Buffalo	Evolution	Di Berardino and Iannuzzi, 1981
		Iannuzzi, 1987
		Berland et al., 1988
	Fertility	King et al., 1980

individual with an abnormal phenotype. Carriers of familial Robertsonian translocation were identified subsequently (Carter et al., 1960; Penrose et al., 1960).

B. Isochromosomes

C.D. Darlington (1939,1940) was the first to describe a chromosome composed of identical arms which he termed an isochromosome. This work with several species of plant meiotic cells (Fritillaria, Lily) provided the foundation for his proposal that isochromosomes arose through centromere misdivision. The incorrect transverse division of the centromere would lead to two products, each metacentric; one composed of the long arms and one of the short arms. Fraccaro (1960) first reported a possible isochromosome in three patients with sex-chromatin positive Turner syndrome. Their karyotypes included one normal X chromosome and one large metacentric chromosome interpreted as an isochromosome Xq.

Chromosome banding techniques allowed for the accurate identification of the chromosomes involved in rearrangements. Caspersson et al. (1971) demonstrated that a t(GqGq) rearrangement was actually a t(21q21q) and a t(DqDq) a t(14q14q). Since then, all acrocentric chromosomes have been found to participate in homologous exchanges (Therman et al., 1989). However, before the availability of restriction fragment length polymorphisms (RFLPs), isochromosomes of the acrocentric chromosomes could not be distinguished from Robertsonian translocations between homologs (Schmutz and Pinno, 1986). Only recently has molecular evidence been

presented in an attempt to show that the two arms of a t(21q21q) are genetically identical (Créau-Goldberg et al., 1987; Priest et al., 1988; Grasso et al., 1989).

II. Incidence of Acrocentric Rearrangements

A. Population incidence and chromosomal aspects of balanced Robertsonian translocations

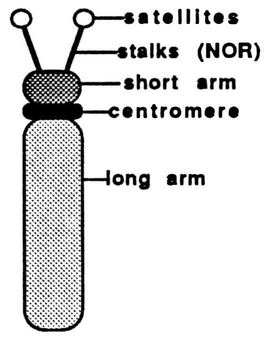
In humans, Robertsonian translocations occur between the acrocentric chromosomes 13, 14, 15, 21, and 22. The incidence of Robertsonian translocations has been documented to be approximately 1 in 1000 individuals based on a survey of 14,069 newborn infants (Hamerton et al., 1975). On the acrocentric chromosomes, the area above the centromere, the short arm, is divided between the proximal short arm, the stalk or secondary constriction where the nucleolus organizer region (NOR) is located, and the satellites. It is thought that there are two types of Robertsonian translocations; monocentric and dicentric (Fig. 1). Monocentric Robertsonian translocations result from breakage in one short arm and one long arm of the participating chromosomes. Dicentric Robertsonian translocations result from breakage in both short arms of the participating chromosomes. The majority of Robertsonian translocations appear to be dicentric (Niebuhr, 1972; Daniel and Lam-Po-Tang, 1976; and Mattei et al., 1979) and devoid of NORs (Mattei et al., 1979; Mikkelsen et al., 1980). In monocentric Robertsonian translocations, the reciprocal short arm (centric) products are usually lost although they have been reported to be retained in a few cases

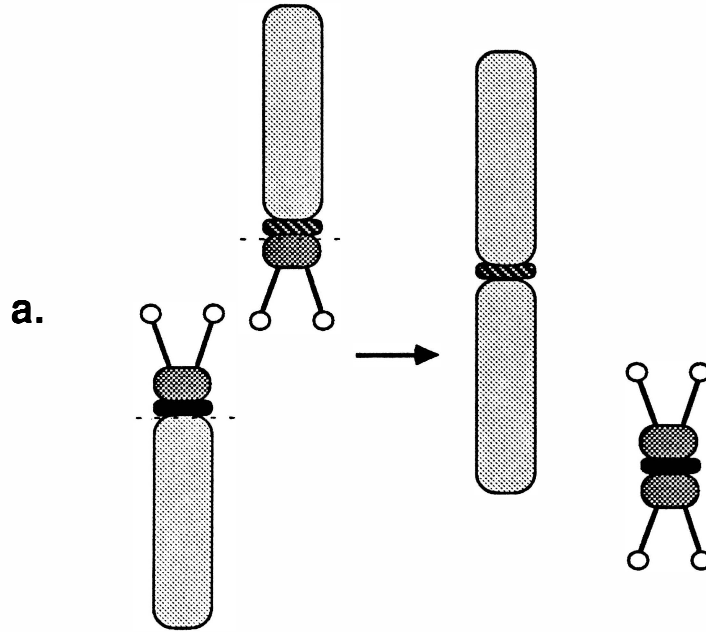
(Palmer et al., 1969; Abeliovich et al., 1985).

Few phenotypic effects are associated with carriers of Robertsonian translocations. The deletion of the short arms and NORs in some Robertsonian translocations does not seem to have a phenotypic effect. However, there may be an association between carriers of Robertsonian translocations and Ph-positive chronic myelocytic leukemia (Engel et al., 1965; Wennstrom et al., 1973; Kohno et al., 1978; Becher et al., 1985 and Becher et al., 1987). In addition, carriers of "balanced" Robertsonian translocations may be at risk for having increased fetal wastage, unbalanced offspring, and infertility or sterility (Stene and Stengel-Rutkowski, 1988). Newborns who are carriers of apparently balanced de novo Robertsonian translocations are at a significant risk for having malformations and/or mental retardation at birth (3.0-5.3%) (Warburton, 1985;1987).

Participation of the acrocentric chromosomes in Robertsonian translocations appears to be non-random (Table 2) (Therman et al., 1989) with 90% of rearrangements between nonhomologs (Mattei et al., 1979). The most common t(DqDq) is t(13q14q) (78%); t(14q21q) is the most common t(DqGq) (79%) and t(21q21q) is the most common t(GqGq) (84%). A possible explanation for the nonrandomness in the acrocentric chromosomes which participate in Robertsonian translocations is that carriers of t(14q21q) are more likely to be ascertained through their trisomic offspring. Likewise, carriers of Robertsonian translocations between homologs may

Fig. 1. Formation of monocentric and dicentric Robertsonian translocations. a. Monocentric Robertsonian translocations result from a break in the short arm of one participating acrocentric chromosome and a break in the long arm of the other chromosome. Two monocentric products result. b. Dicentric Robertsonian translocations result from breaks in both short arms of the participating chromosomes. The resulting short arm acentric product is usually lost in subsequent cell divisions.


satellites
stalks (NOR)
short arm
centromere
long arm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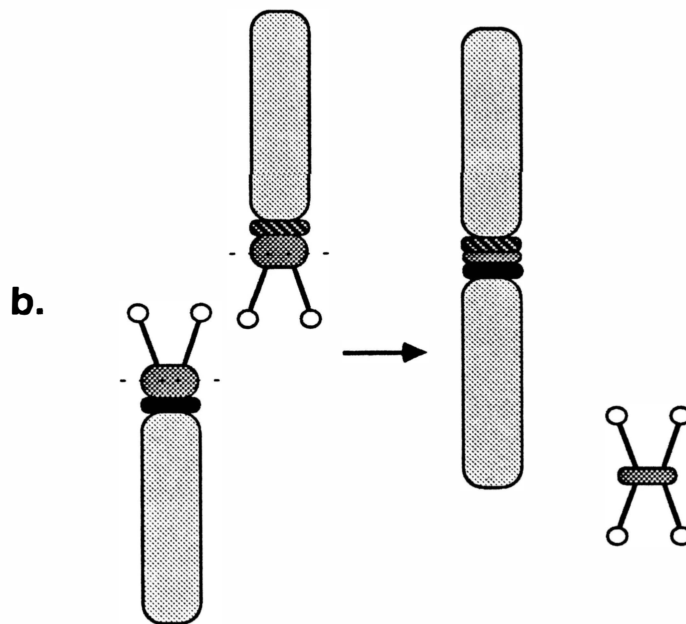

b.

be over-represented because of their ascertainment through multiple miscarriages, trisomic offspring, or an abnormal phenotype. Although rare, there have been a few individuals reported with two Robertsonian translocations (Marsden et al., 1966; Rockman-Greenberg et al., 1982; Martinez-Castro et al., 1984; Morgan et al., 1985 and Eklund et al., 1988).

B. Incidence and cytogenetic aspects of unbalanced Robertsonian translocations

The most common syndromes associated with unbalanced Robertsonian translocations are Down syndrome and Patau syndrome. Approximately 5% of cases of Down syndrome are caused by a translocation, the majority of which are Robertsonian (>95%) (de Grouchy and Turleau, 1984). Thus, about 200 children are born each year in the United States with translocation Down syndrome (Pulliam and Huether, 1986). The majority of cases of translocation Down syndrome are de novo (69%) and the remaining cases are due to a familial translocation (31%) (de Grouchy and Turleau, 1984). Table 3 shows the proportion of t(Dq21q) and t(21qGq) found in patients with translocation Down syndrome (adapted from de Grouchy and Turleau, 1984). The proportion of t(14q21q) and t(21q21q) are about equal. However, the vast majority of t(21q21q) are de novo and therefore represent the largest class of de novo rearrangements in Down syndrome.

The proportion of cases of t(21q21q) that result from an i(21q) are unknown. Two cases have been reported in which a patient with Down syndrome was mosaic 45,rob(15;21)/ 46,i(21q)

Table 2. Nonrandom participation of acrocentric chromosomes in Robertsonian translocations[a]

	13	14	15	21	22
13	43 (2.9)[b]	-	-	-	-
14	479 (32.6)	9 (0.6)	-	-	-
15	28 (1.9)	34 (2.3)	24 (1.6)	-	-
21	32 (2.2)	447 (30.4)	46 (3.1)	245 (16.7)	-
22	11 (0.7)	18 (1.2)	9 (0.6)	30 (2.0)	16 (1.1)

[a] from Therman et al., 1989
[b] percent total observed in 1471 subjects studied

(Atkins and Bartsocas, 1974; Vianna-Morgante and Nunesmaia, 1978). In both cases, one possible explanation for the i(21q) was its derivation from the rob(15;21) through misdivision of the centromere.

Approximately 20% of cases of Patau syndrome result from a translocation and 10% are accounted for by an unbalanced de novo Robertsonian translocation (Hook, 1978; Pérez-Castillo and Abrisqueta, 1978). The translocations are mostly t(13qDq) with the majority of these being t(13q14q) (de Grouchy and Turleau, 1984). A few cases of non-t(13q14q) Patau syndrome have been reported [i.e. one t(13q21q) (Pérez-Castillo and Abrisqueta, 1978), two t(13q22q) (Abe et al., 1975; Daniel and Lam-Po-Tang, 1976) and one t(13q15q) (Mori et al., 1985)].

Table 4 shows the likelihood that a translocation ascertained from a proband with translocation Down syndrome or Patau syndrome is a new mutation (adapted from Hook, 1981). Nearly all cases of t(21q21q) and t(13q13q) are new mutations. Collectively, about 1/10,000 pregnancies results in an unbalanced Robertsonian translocation and almost all are de novo (Hook, 1984).

C. Estimates of mutation rates for de novo Robertsonian translocations.

Mutation rates for Robertsonian translocations have been estimated from literature surveys of spontaneous abortions (Jacobs, 1981) or livebirths (Polani et al., 1965; Jacobs, 1981), newborns with translocation Down syndrome in Japan (Kikuchi et al., 1969), New York (Hook and Albright, 1981;

Table 3. The proportion of t(Dq21q) and t(21qGq) in probands with translocation Down syndrome.[a]

Rearrangement	% Total	% *de novo*	% Familial
t(Dq21q)	54.2	55.0	45.0
t(13q21q)	11.9		
t(14q21q)	31.7		
t(15q21q)	10.6		
t(21qGq)	40.9	96.0	4.0
t(21q21q)	34.1		
t(21q22q)	6.8		
non-rob	4.9	22.0	78.0

[a] Adapted from de Grouchy and Turleau (1984) in which 4,760 cases were studied

Table 4. Likelihood that a translocation ascertained from a proband with translocation Down syndrome or Patau syndrome is a new mutation.[a]

Rearrangement	Likelihood of de novo rearrangement
t(13q21q)	0.30
t(14q21q)	0.69
rea(21q21q)	0.93
t(21q22q)	0.50
rea(13q13q)	0.90
t(13q14q)	0.45

[a] Adapted for Hook, 1981

Hook, 1981), or Ohio (Pulliam and Huether, 1986), and livebirths with translocation Patau syndrome (Hook, 1981). Since it is impossible to distinguish isochromosomes 21 and 13 from Robertsonian translocations, the proportion of de novo rearrangements resulting from isochromosomes is unknown. Therefore, all events leading to Robertsonian-type rearrangements are considered together for calculating mutation rates. As shown in Table 5, the mutation rate estimates for unbalanced t(DqGq) and t(GqGq) are in close agreement between the five surveys of livebirths with translocation Down syndrome ($\sim 1X10^{-5}$) (Polani et al., 1965; Kikuchi et al., 1969; Hook and Albright, 1981; Hook, 1981; Pulliam and Huether, 1981) and with Jacobs (1981) for unbalanced livebirths with t(21qGq) for which the majority are t(21q21q). The mutation rate estimates from surveys of spontaneous abortions (Jacobs, 1981) shows about a 100-fold increase in the total mutation rate in these cases as compared to livebirths, with the majority accounted for by t(DqDq) rearrangements.

D. Parental age, temporal changes, and mutagen exposures

There have been numerous studies documenting the advanced maternal age association in trisomy 21 (for a review see Mikkelson, 1971). The first study of parental age effects in translocation Down syndrome is that of Penrose (1962) in which he found that for t(Dq21q), there was no difference in maternal or paternal age from the control group. However, the paternal age for t(21qGq) was significantly increased over

Table 5. Comparison of mutation rate estimates for Robertsonian translocations (expressed as mutations/gamete/generation).

Source		Balanced				Unbalanced			
		D/D	D/G	G/G	Total	D/D	D/G	G/G	Total
Polani et al. (1965)		*	*	*	*	*	$0.6X10^{-5}$	$1.4X10^{-5}$	$2.0X10^{-5}$
Kikuchi et al. (1969)		*	$0.68X10^{-5}$	$0.19X10^{-5}$	$0.87X10^{-5}$	*	$1.01X10^{-5}$	$1.14X10^{-5}$	$2.5X10^{-5}$
Jacobs (1981)	livebirths	$0.24X10^{-4}$	$0.16X10^{-4}$	*	*	$1.3X10^{-5}$	*	$1.0X10^{-5}$	$2.3X10^{-5}$
	spontaneous abortions	*	*	*	*	$16.0X10^{-4}$	$4.4X10^{-4}$	$2.6X10^{-4}$	$23.0X10^{-4}$
	All conceptions	$0.20X10^{-4}$	$0.16X10^{-4}$	*	$0.57X10^{-4}$	$2.51X10^{-4}$	$0.66X10^{-4}$	$0.48X10^{-4}$	$3.54X10^{-4}$
Hook and Albright (1981)		*	*	*	*	*	$1.1X10^{-5}$	$1.4X10^{-5}$	$2.5X10^{-5}$
Hook (1981)		*	*	*	*	$0.8X10^{-5}$	$1.0X10^{-5}$	$1.4X10^{-5}$	$3.2X10^{-5}$
Pulliam and Huether (1981)		*	*	*	*	*	$0.9X10^{-5}$	$1.3X10^{-5}$	$2.2X10^{-5}$

Notes:
* = values not given in original report.
Except for Jacobs (1981), all D/D were 13/13, all D/G were D/21 and all G/G were G/21 with the majority of G/21 being 21/21.
All types of Robertsonian translocations were accounted for by Jacobs (1981).

the control population. In general, translocations are found more often in children with Down syndrome born to younger mothers (Mikkelsen, 1971). Since these early reports, several studies have been conducted to examine the effects of parental age on the formation of unbalanced de novo Robertsonian translocations. The majority of these cases were patients with Down syndrome. These studies are summarized in Table 6. For de novo t(Dq21q), all studies found no significant difference in the mean paternal age as compared to controls (Kikuchi et al., 1969; Matsunaga and Tonomura, 1972; Hook, 1984; Pulliam and Huether, 1986). Although two studies found no significant difference in mean maternal age for t(Dq21q) with controls (Kikuchi et al., 1969; Hook, 1984), two studies found a significant decrease in maternal age in this category (Matsunaga and Tonomura, 1972; Pulliam and Huether, 1986). Although not significant, the mean paternal age was slightly higher in de novo t(21qGq) cases as compared to controls in three of four studies (Kikuchi et al., 1969; Matsunaga and Tonomura, 1972; Hook, 1984). Two studies found a significant increase in mean maternal age for t(21qGq) (Matsunaga and Tonomura, 1972; Hook, 1984). These findings suggest that there is a parental age effect in translocation Down syndrome; specifically, there may be an association between decreased maternal age and de novo t(Dq21q) and between increased maternal age and de novo t(21qGq). There does not appear to be any effect related to paternal age, contrary to previous reports (Penrose, 1962). Unfortunately, few studies have been

conducted on the parental age effects and non t(Dq21q) or t(21qGq) Robertsonian translocations. For de novo (13qDq) cases, Hook (1984) found no parental age effect, maternal or paternal. In addition, Jacobs (1981) found no association between parental age and 8 de novo structural rearrangements (at least 3 were Robertsonian translocations).

Three studies have investigated the temporal changes or fluctuation rates for de novo translocation Down syndrome (Hook, 1978; Hook and Albright, 1981; Pulliam and Huether, 1986). Only one data set (Hook and Albright, 1981) exhibited a change in mutation rates from year to year. The increase in mutation rates from 1973-1977 may have reflected 1) an increase in ascertainment during these years; although amniocentesis was not widely used until after this period, 2) a real increase in the number of mutations that occurred, or 3) a decrease in the number of abnormal pregnancies that were spontaneously aborted because of an unbalanced translocation. Specific environmental, occupational, demographic or medical factors could not be identified to explain the increased occurrence.

Environmental factors predisposing to structural abnormalities, specifically de novo Robertsonian translocations, have been examined (Hook et al., 1983). Of 71 pregnancies studied for exposure to radiation and 65 studied for exposure to a drug or chemical, none of the fetuses were found to carry a Robertsonian translocation. To support the finding that no identified environmental factors

Table 6. Mean parental age for unbalanced Robertsonian translocations

Rearrangement	Mean Age: Maternal (control)	Mean Age: Paternal (control)	Source
t(Dq21q)	25.5±0.51[a] (27.1)	29.9±0.90 (30.4)	Matsunaga and Tonomura (1969)
	25.7±0.84 (27.2±1.4)	30.2±1.11 (30.6±1.6)	Kikuchi et al. (1972)
	25.5±5.4 (25.6±0.2)	28.7±7.0 (28.3±4.9)	Hook (1984)
	21.6±1.18[a] (24.6)	24.2±1.74 (27.5)	Pulliam and Huether (1986)
t(21qGq)	30.8±0.78[b] (27.1)	28.5±0.71 (30.4)	Matsunaga and Tonomura (1969)
	28.8±0.94 (27.1±1.4)	31.4±0.99 (30.3±1.1)	Kikuchi et al. (1972)
	27.2±5.7[b] (25.7±0.2)	30.8±8.0 (30.0±5.1)	Hook (1984)
	24.4±1.29 (24.6)	26.4±1.33 (27.5)	Pulliam and Huether (1986)

[a] Significant decrease in mean age as compared to control group.
[b] Significant increase in mean age as compared to control group.

have been associated with the formation of Robertsonian translocations in humans, Hecht (1976) found that these rearrangements rarely occurred following radiation or chemical exposure and did not appear to be inducible.

III. Reproductive fitness

A. Carriers of balanced Robertsonian translocations

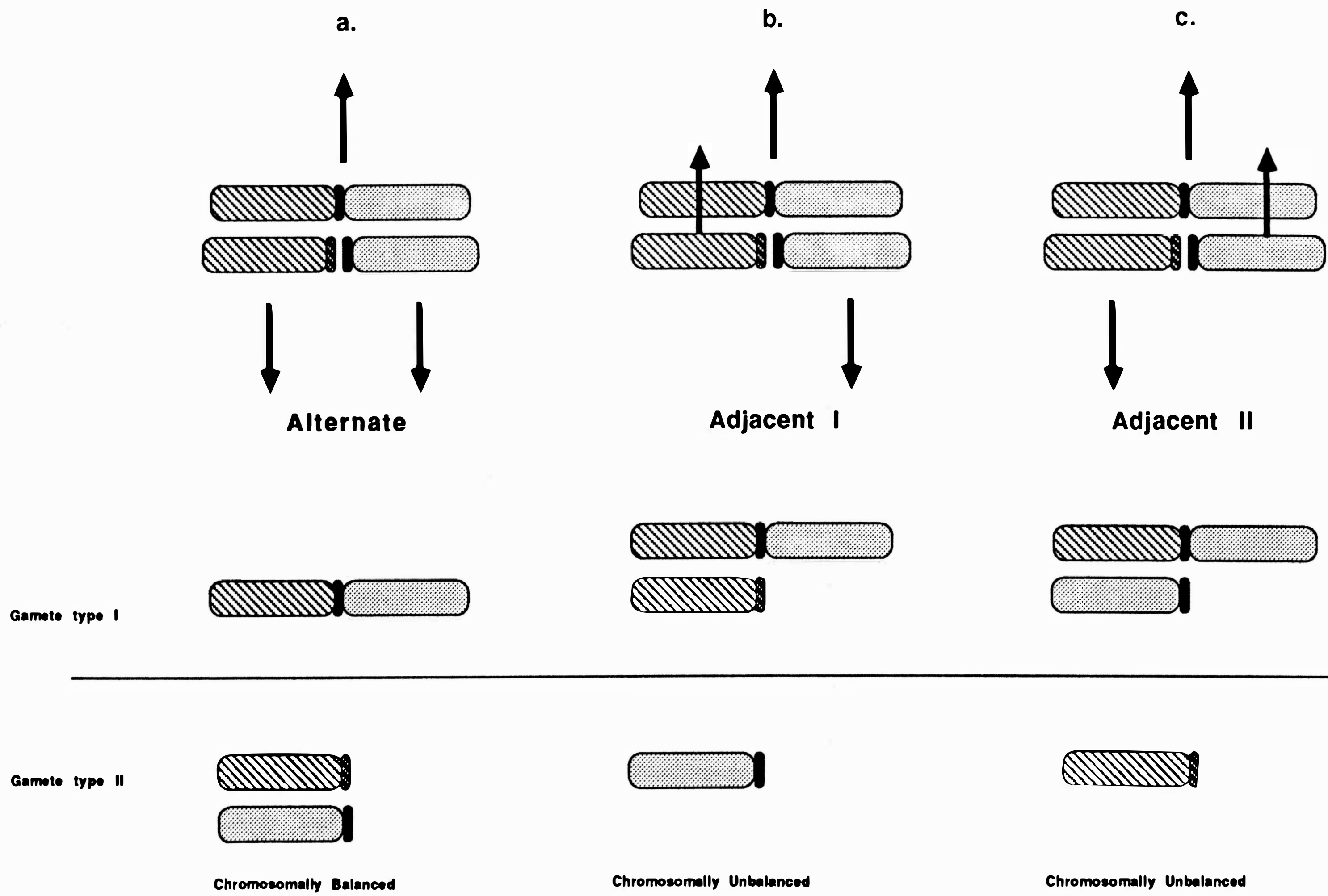
Carriers of Robertsonian translocations have 45 chromosomes and although there is loss of short arm chromatin, the carrier is considered balanced. Balanced carriers of Robertsonian translocations can be ascertained through prenatal testing, unbalanced offspring, multiple miscarriages or infertility. Their recurrence risk for fetal wastage or abnormal offspring may be inferred from their mode of ascertainment since translocations that are more likely to result in chromosomally abnormal offspring are more likely to be ascertained (Stene and Stengel-Rutkowski, 1988). Clearly, the risk for unbalanced offspring depends on the chromosomes involved in the Robertsonian translocation. A balanced carrier of a Robertsonian translocation between homologous chromosomes (or a carrier of an acrocentric isochromosome) will have only unbalanced offspring. Carriers of nonhomologous Robertsonian translocations [i.e. t(14q21q)] have three possible segregation patterns as illustrated in Figure 2. Alternate segregation produces gametes that will either become chromosomally normal offspring or balanced carriers like their carrier parent. Adjacent segregation produces only unbalanced gametes which result in monosomic or

tertiary trisomic offspring.

It has long been documented that the segregation ratios from male carriers of balanced Robertsonian translocations are nonrandom (Hamerton, 1968). There is a significant excess of balanced carriers over chromosomally normal offspring (Hamerton, 1971). The segregation ratios for balanced carrier offspring have been reported to range from 0.66 (Nielsen and Rasmussen, 1976) to 0.55 (Harris et al., 1979). Additionally, there is a significant deficiency of unbalanced offspring from male carriers (Hamerton, 1971; Nielsen and Rasmussen, 1976; Harris et al., 1979). These observations may be explained in three ways. First, there may be a selective advantage for translocation carrier sperm over chromosomally normal sperm giving rise to more carrier offspring. Second, there may be gametic selection against unbalanced sperm which would lower the frequency of unbalanced offspring. Finally, there may be early selection against unbalanced embryos resulting from paternally inherited translocations.

Since the reproductive risks for carriers of Robertsonian translocations depend on the specific chromosomes involved, the various types of rearrangements [DqDq, DqGq and GqGq] will be considered separately. The risk for unbalanced offspring for both male and female carriers of DqDq Robertsonian translocations is very low (<1%) (Stene and Stengel-Rutkowski, 1988). The greatest risk is for Patau syndrome through adjacent segregation. In addition, there have been several

Fig. 2. Segregation patterns for a balanced carrier of a Robertsonian translocation. a. Alternate segregation results in balanced gametes; chromosomally normal and translocation bearing. The gametes will produce chromosomally normal and balanced carrier offspring. b. Adjacent I segregation of nonhomologous centromeres and c. adjacent II segregation of homologous centromeres. Adjacent segregation produces only unbalanced gametes, disomic and nullisomic, which result in trisomic and monosomic offspring, respectively.


a.
b.
c.
Alternate
Adjacent I
Adjacent II
Gamete type I
Gamete type II
Chromosomally Balanced
Chromosomally Unbalanced
Chromosomally Unbalanced

reports of DqDq carriers having children with trisomy 21 (Hamerton et al., 1963; Hamerton, 1968; Fernhoff et al., 1976; Harris et al., 1979; Lindenbaum et al., 1985; Uchida and Freeman, 1986; Gallego and Coco, 1988). The presence of a t(DqDq) is thought to disrupt the normal pairing process during meiosis of the chromosomes not involved in the translocation (Grell and Valencia, 1964). This inter-chromosomal effect may contribute to nondisjunction and may result in aneuploid gametes (Lindenbaum et al., 1985). However, this assumes that the DqDq carrier was the parent who contributed the extra chromosome 21. Only one study has examined the parental origin of the extra chromosome 21 and found that it was contributed by the non-Robertsonian translocation carrier parent (Uchida and Freeman, 1986). Although the risk of trisomy 21 to DqDq translocation carriers may be as much as 2% (Mikkelsen, 1971), several studies have failed to find this association in spontaneous abortions or livebirths (Boué and Boué, 1973; Nielsen and Rasmussen, 1976; Evans et al., 1978; Neri et al., 1983; Campana et al., 1986; Schwartz et al., 1986). Thus, it is unclear if a relationship exists between trisomy 21 and carriers of DqDq translocations and this association could be by chance.

Infertility has been reported occasionally in male carriers of t(13q14q) (Walker and Harris, 1962; Yunis et al., 1964; McIlree et al., 1966; Wilson, 1971; de Kretser et al., 1972; Fracarro et al., 1973). Decreased fertility has been attributed to oligospermia in some males (McIlree et al.,

1966; de Kretser et al., 1972; Fraccaro et al., 1973). However, one of the largest studies of carriers of t(13q14q) found no decrease in fertility among male carriers (Nielsen and Rasmussen, 1976).

In an attempt to uncover the causes of infertility in carriers of Robertsonian translocations, a study of rDNA levels was conducted (Guanti et al., 1980). Carriers of Robertsonian translocations may be expected to have less rDNA because of a loss during the formation of the translocation. In order to test this, hybridization of rDNA to rRNA was carried out for twelve infertile male carriers of Robertsonian translocations, six fertile male carriers, and six karyotypically normal males. There was no significant difference in the mean hybridization levels, thus not supporting the hypothesis that infertility is related to decreased levels of rDNA.

A new approach for studying the segregation of t(13q14q) comes from analysis of sperm karyotypes (Pellestor et al., 1987; Martin, 1988). Each study examined one carrier male. Both studies found an equal distribution of karyotypically balanced and normal sperm. Neither study demonstrated an inter-chromosomal effect of the 13q14q translocation and aneuploidies of chromosomes unrelated to the translocation (i.e. trisomy 21). Although few subjects have been studied, both investigations demonstrated low unbalanced hyper-haploid sperm complements for chromosome 13 (2.5% and 10%) and chromosome 14 (2.5% and 4%) (Pellester et al., 1987 and

Martin, 1988, respectively).

The risk for unbalanced offspring and fetal wastage is higher for carriers of t(DqGq) than for t(DqDq) since the majority of DqGq are Dq21q and contribute significantly to the occurrence of translocation Down syndrome (Stene and Stengel-Rutkowski, 1988). The segregation ratio for balanced DqGq carriers is 0.57 and differs significantly from 0.50 (Stene, 1970). The risk for having unbalanced offspring is different for male and female carriers. Female carriers of t(DqGq) have about a 10.1% risk for unbalanced liveborn and a 14.5% risk for an unbalanced fetus detected by amniocentesis (Stene and Stengel-Rutkowski, 1988). Male carriers are at a much lower risk for unbalanced offspring (Neri et al., 1983). In this study of 58 carriers of t(DqGq), no unbalanced offspring were born to male carriers.

Although all studies agree that carriers of t(14q21q) are at risk for Down syndrome, one report noted a significant increased risk of Down syndrome for carriers of t(13q21q) over t(14q21q) (Daniel et al., 1980). However, the number of cases was too small (4 families) to see an effect. In one study, investigators examined the sperm of a male carrier of a t(14q21q) and found a significantly greater number of karyotypically normal sperm as compared to balanced sperm (Balkan and Martin, 1983). Additionally, 4% (1/24) of the sperm complements carried an extra chromosome 21. A similar study was done on a carrier of a t(15q22q) (Syme and Martin, 1989). They noted a slightly greater number of chromosomally

normal sperm as compared to chromosomally balanced sperm and there was no evidence for an inter-chromosomal effect for aneuploidies unrelated to the translocation.

For balanced carriers of GqGq translocations, only t(21q22q) will be considered since all offspring of t(21q21q) and t(22q22q) will be unbalanced. The risk to female carriers of t(21q22q) for unbalanced offspring is 8.9% for unbalanced liveborn and 15.8% for unbalanced fetuses at amniocentesis (Stene,1970; Stene and Stengel-Rutkowski, 1988). Again, the risk for male carriers is very low. Sperm from a male heterozygous for a t(21q22q) has been examined (Syme and Martin, 1988). They found a slight increase in the number of chromosomally normal sperm as compared to chromosomally balanced sperm and did not see any evidence for an inter-chromosomal effect for non-translocation related aneuploidies.

In summary, the risk of unbalanced offspring to carriers of Robertsonian translocations appears to depend on the sex of the carrier and the specific chromosomes involved in the rearrangement. Some other factors that have not been studied extensively include: 1) The segregation patterns of dicentric versus monocentric Robertsonian rearrangements; 2) the effects of retention of specific centromeres in monocentric rearrangements; and 3) the size discrepancy between two chromosomes involved in a Robertsonian translocation and the contribution to malsegregation. In reference to points one and two, the particular functional centromere may lead to the segregation patterns seen in certain Robertsonian

translocations. For example, carriers of t(13q21q) rarely have children with Patau syndrome but are at a significant risk for having children with Down syndrome (Daniel et al., 1980). The majority of t(13q21q) may retain the centromere 13 as the functional centromere which may lead to a predisposition for adjacent I segregation of the two nonhomologous centromeres resulting in Down syndrome (Fig. 2). The adjacent II type of segregation pattern is much rarer and may explain the rarity of Patau syndrome in the children of carriers of t(13q21q). The third point may be clarified by examining the contribution of unbalanced offspring from carriers of t(13q14q) as compared to t(14q21q). The unequal sized chromosomes involved in the translocation may contribute to improper meiotic pairing, malsegregation and result in unbalanced gametes (Hamerton, 1963). However, when two sperm studies are compared, a carrier of a t(13q14q) had 26.5% (31/117) unbalanced sperm complements while a carrier of a t(14q21q) had 12.5% (3/24) unbalanced complements (Martin, 1988 and, Balkan and Martin, 1983, respectively), thus not lending support to the hypothesis of malsegregation resulting from unequal sized chromosomes. Further studies into the molecular structure of Robertsonian translocations may uncover specific characteristics of certain Robertsonian translocations that contribute to fetal wastage and unbalanced offspring.

B. Recurrence risk for de novo acrocentric rearrangements

The recurrence risk for de novo rearrangements may result from 1) a true recurrence of the de novo event because of a genetic predisposition; 2) recurrence from chance alone or 3) parental mosaicism. Waxmann and Arakaki (1966) were the first to report a GqGq mosaic carrier mother and her three children with translocation Down syndrome who were fathered by two different males. Wilroy et al. (1969) reported two siblings with "de novo" GqGq Down syndrome in which the mother was found subsequently to be mosaic (Wilroy et al., 1978). In addition, Steinberg et al. (1984) found parental mosaicism in four previously reported de novo translocation Down syndrome cases. Hall (1985) reported a 1% maternal mosaicism in one case and cautioned against giving low recurrence risk figures without exploring parental mosaicism. Although gonadal mosaicism can rarely be excluded, the risk for having a second child with de novo translocation Down syndrome appears to be less than 1% in a study of 76 families (Gardner and Veale, 1974) to 2% in a study of 112 families (Steinberg et al., 1984). However, all studies agree that parents who have had a child with a de novo translocation should be offered prenatal testing in subsequent pregnancies (Gardner and Veale, 1974; Schmidt and Nitowsky, 1977; Garver et al., 1982; Steinberg et al., 1984).

The recurrence risks for de novo t(21q21q) versus i(21q) have not been examined. Additionally, because of the rare occurrence, the recurrence risk for non-t(21q21q) de novo

rearrangements has not been examined.

IV. Causes of Acrocentric Rearrangements

A. The nucleolar organizer region

In humans, the nucleolar organizer regions (NORs) are located in the secondary constriction of the five pairs of acrocentric chromosomes: 13, 14, 15, 21 and 22 (Ferguson-Smith and Handmaker, 1961). The NOR is the location of the tandemly arranged genes coding for the 18S and 28S ribosomal RNA (Evans et al., 1974). Based on ^{3}H-rRNA _in situ_ hybridization studies, the amount of rDNA present differs between the acrocentric chromosomes and between individuals (Evans et al., 1974). Additionally, the amount of rDNA is stable within an individual and heritable (Evans et al., 1974).

By employing an ammoniacal silver staining technique, Goodpasture et al. (1976) confirmed that the NOR was located in the stalk region (secondary constriction) of the acrocentric chromosomes. The ammoniacal silver stain was shown to stain the acidic proteins that surround the active NOR (Schwarzacher et al., 1978), thus developing a measurement of NOR activity. Morton et al. (1983) demonstrated that total NOR score, by silver staining, correlated positively with the incorporation of ^{3}H-uridine into nucleolar rRNAs. In addition, they were able to show heritability of NOR activity (transcription) within monozygotic twins (Morton et al., 1981). Other investigators have also demonstrated the heritability of NOR expression (Markovic et al., 1978; Taylor

and Martin-DeLeon, 1981; Zakharov et al., 1982a; Zakharov et al., 1982b). Recently, investigators have shown that transcriptionally active NORs are less methylated and more sensitive to DNase I digestion than inactive NORs (Ferraro and Prantera, 1988).

The number of positively staining NORs in individuals has been shown to vary between four and ten with a mean of about eight (Bloom et al., 1976; Mikelsaar et al., 1977; Ray and Pearson, 1979; Jackson-Cook, 1985). The frequency of positive NORs was lowest for chromosome 22 (Mikelsaar et al., 1977; Zakharov et al., 1982b) and highest for chromosome 21 (Zakharov et al., 1982b).

The role of the NOR in chromosomal abnormalities was first suggested by Ohno et al. (1961). They hypothesized that during the formation of the nucleolus and transcription of rDNA, when the acrocentric chromosomes participate in satellite associations, breakage and exchange could occur between the acrocentric chromosomes giving rise to Robertsonian translocations. Evidence for NOR involvement in Robertsonian translocations comes from Miller et al. (1978) and Di Berardino and Iannuzzi (1981) in mice and cattle, respectively. The mouse is an exceptional model for studying Robertsonian translocation formation because all 20 pairs of chromosomes are acrocentric and each chromosome contains equivalent amounts of highly repetitive satellite DNA. However, only three to six pairs of chromosomes have active NORs. Miller et al. (1978) found that the chromosomes with

active NORs were more likely to be involved in Robertsonian translocations than the chromosomes without NORs or inactive NORs. Additionally, about 30% of the rearrangements were homologous Robertsonian translocations or isochromosomes. The proportion of NOR bearing chromosomes resulting in "isochromosomes" was greater than expected if all chromosomes were equally likely to participate. The "isochromosomes" could have resulted from "centric fusion" of homologous chromosomes or through misdivision of the centromere.

Robertsonian translocation formation is the primary cause of karyotypic evolution in cattle (Di Berardino and Iannuzzi, 1981). One of the most investigated examples is the evolutionary relationship between two types of buffalo; Swamp (2N=48) and Murrah (2N=50). Di Berardino and Iannuzzi (1981) demonstrated that fusion took place between chromosomes 4 and 9 in the Murrah buffalo to give rise to the karyotypic findings in the Swamp buffalo. Evidence for NOR involvement in this translocation is provided by 1) the Murrah buffalo chromosome 4, which is NOR-positive, is one of the chromosomes in the Robertsonian translocation of the Swamp buffalo; and 2) the finding of an apparent loss of a NOR through a centric fusion in the Swamp buffalo, who have only five pairs of NOR positive chromosomes, as opposed to the six NOR-positive pairs in the Murrah buffalo.

The role of the NOR in human acrocentric rearrangements comes from a series of studies which explored exchanges of ribosomal genes between nonhomologous chromosomes (Arnheim et

al., 1980; Krystal et al., 1981) and the association of ribosomal genes from nonhomologous chromosomes in the nucleolus (Mirre et al., 1980; Stahl et al., 1983). Restriction enzyme analysis of the ribosomal genes between humans and five species of apes revealed concerted evolution. In this case, concerted evolution refers to the fact that the ribosomal genes on nonhomologous chromosomes within and between species are too similar to have arisen independently (Arnheim et al., 1980). The variability seen between chromosomes could be explained by unequal sister chromatid exchanges within a chromosome and crossing over between nonhomologous chromosomes (Krystal et al., 1981). Studies of the ultrastructure of the nucleolus of human oocytes and spermatocytes reveal that ribosomal genes belonging to several acrocentric chromosomes associate in the same nucleolus (Mirre et al., 1980; Stahl et al., 1983). During this association, it has been hypothesized that breakage and erroneous reunion could result in Robertsonian translocations. Since the ribosomal genes intermingle, but not necessarily the entire pericentromeric regions, more dicentric translocations would be expected. However, homologous areas of the short arms and pericentromeric regions between nonhomologous chromosomes may facilitate pairing and exchange, resulting in both dicentric and monocentric translocations.

B. The pericentromeric regions

The pericentromeric regions of human chromosomes contain a class of DNA known as satellite DNA. Satellite DNA refers

to tandemly repeated DNA families that can be separated from the rest of the DNA by a CsCl gradient (Lewin, 1983). The function of satellite DNA is unknown at this time although it has been postulated to be involved in chromosomal organization and structure, gene regulation, and maintaining chromosome pairing and order within the nucleus (Devine et al., 1985). There are several families of satellite DNA: alpha and satellite I-IV (Choo et al., 1989). The order of the repeating units of satellite DNA is characteristic of individual chromosomes and is thought to arise through mutation and crossing over between homologous chromosomes (Devilee et al., 1986). However, other evidence for only the acrocentric chromosomes indicates that satellite DNA has been dispersed over these chromosomes through unequal crossing over between nonhomologs (Kurnit, 1979).

Many DNA sequences have been isolated that hybridize to all five human acrocentric chromosomes, thus displaying sequence homology between the acrocentrics (Kurnit et al., 1984; Willard, 1985; Devine et al., 1985; Kurnit et al., 1986). Recently, subfamilies of α-satellite DNA sequences have been shown to hybridize to specific acrocentric chromosomes. Two chromosome-specific subfamilies have been shown to hybridize to only chromosomes 13 and 21 (Devilee et al., 1986; Jørgensen et al, 1987). Additionally subfamilies have been shown to hybridize specifically to chromosomes 14 and 21 (Choo et al., 1988); chromosome 14 (Waye et al., 1988); chromosome 22 (McDermid et al., 1986); and chromosomes 14 and

22 (Jørgensen et al., 1988). The emergence of these chromosome specific α-satellite DNA sequences have prompted Choo and co-workers to propose their model of Robertsonian translocation formation through specific acrocentric short arm domains (Choo et al., 1988; 1989).

The model proposed by Choo et al. (1988; 1989) considers the following information: 1) α-satellite DNA sequences have been identified that are common to all acrocentric chromosomes and unique to some; 2) acrocentric chromosomes participate nonrandomly in Robertsonian translocations; and 3) acrocentric chromosomes participate in satellite associations. Two assumptions are made: First, exchanges can occur between nonhomologous acrocentric chromosomes and second, the orientation of the domains on chromosome 14 are inverted compared to chromosomes 13 and 21 (Therman, 1980). The homology and repetitive nature of the satellite domains between the acrocentric chromosomes, specifically chromosomes 13, 14 and 21, allows for homologous pairing between nonhomologs. Additionally, if the sequences on chromosome 14 are inverted, this would explain the high frequency of t(13q14q) and t(14q21q) *and the rare occurrence of* t(13q21q); t(13q14q) and t(14q21q), 2 of 15 possible translocations, account for the majority of cases (Therman et al., 1989). Furthermore, the short arm location of these domains is consistent with the site of breakage within the majority of Robertsonian translocations. Breaks are thought to occur in the short arms since the majority of Robertsonian

translocations are dicentric and devoid of NOR material (Mattei et al., 1979). Although chromosomes 14 and 22 and chromosomes 13 and 21 share common domains, the sequences on these chromosomes are thought to be in the same orientation, as opposed to being inverted, which explains the low frequency of these particular Robertsonian translocations (1.2% and 2.2%, respectively) (Therman et al., 1989). However, the α-satellite domains that have been shown to be present in all of the acrocentric chromosomes could explain the occurrence of all 15 possible Robertsonian translocations seen in the population (Choo et al., 1988).

C. Satellite associations

The acrocentric chromosomes participate in a phenomenon termed satellite association. First reported by Ferguson-Smith and Handmaker (1961), satellite associations are the result of nucleolus formation and rDNA transcription in the previous cell cycle. This observation has led to the hypothesis that during nucleolus formation, breakage and erroneous exchange leads to Robertsonian translocation formation (Ferguson-Smith and Handmaker, 1961; Ohno et al., 1961). Therefore, satellite associations, measured at metaphase, could be a measure of each chromosome's participation in nucleolus organization in the previous interphase.

The acrocentrics have been found to participate in satellite associations randomly (Cohen and Shaw, 1967; Curtis, 1974; Mattei et al., 1976; Jacobs et al., 1976, Therman et

al., 1989) and nonrandomly (Patil and Lubs, 1971; Galperin-Lemaitre et al., 1977; Ray and Pearson, 1979). Patil and Lubs (1971) found that chromosomes 13, 14 and 21 participated most frequently while Galperin-Lemaitre et al. (1977) found that chromosomes 13 and 21 preferentially associated. Additionally, Ray and Pearson (1979) observed that 15-22 associations were higher than expected.

Several studies have identified factors that may influence the formation of satellite associations. Ribosomal DNA content is one such factor which has been investigated. Evans et al. (1974) showed with ^{3}H-rRNA hybridization studies that acrocentric chromosomes in association do not exhibit more rRNA hybridization than the chromosomes not in association. However, with few exceptions, Warburton et al. (1976) found a significantly positive correlation between the number of rDNA gene copies and a tendency for satellite associations. It is possible that satellite associations are more frequent between silver-positive (active) NOR bearing chromosomes. Tantravahi et al. (1976), Di Lernia et al. (1980) and Morton et al. (1981) found that the amount of silver staining was increased on the associated acrocentric chromosomes. However, silver-negative chromosomes 22 participated in satellite associations as frequently as the silver-positive chromosomes (Tantravahi et al., 1976). Furthermore, Tantravahi et al. (1976) found that in the Gorilla, the acrocentrics that lacked NORs (and rDNA) also participated in satellite associations. Therefore, satellite

associations are not a simple function of rDNA content or NOR activity.

Other factors which may influence satellite associations include the length of the short arm, short arm heteromorphisms, the length of the stalk and the size of the satellites. Zankl and Zang (1974) found that acrocentric chromosomes with elongated short arms had lower association frequencies than the other acrocentric chromosomes. Jacobs et al. (1976) observed no correlation between any morphological heteromorphisms and satellite associations. Additionally, no relationship could be established between R-band variants of the short arms and the frequency of acrocentric associations (Balicek et al., 1982). Acrocentric chromosomes with long stalk lengths have been found to associate more frequently than their "normal" homologs (Schmid et al., 1974; Miller et al., 1977; de Capoa et al., 1978; Di Lernia et al., 1980). Acrocentric chromosomes with large satellites have been found to have increased satellite associations in one study (Zankl and Zang, 1974) and no difference from the other acrocentrics in another (Di Lernia et al., 1980). Finally, the frequency of satellite associations have not been found to differ between males and females (Zang and Back, 1968; Mattei et al., 1976; Ray and Pearson, 1979) although there may be an increase in associations for individuals over age 40 (Mattei et al., 1976). However, satellite associations have been found to be highly heritable (Phillips, 1975; Yip and Fox, 1981).

Satellite associations have been studied in parents and their offspring who have trisomy 21. Cooke and Curtis (1974) found no increase in satellite associations in the parents as compared to the control individuals. However, the parental origin of the nondisjunctional event was not studied. In two studies where the parent in whom the nondisjunction occurred was determined, one study found an increase in satellite associations of chromosomes 21 and 14 and specifically 21-21 associations (Hansson and Mikkelsen, 1978) while the other noted an increase in all satellite associations in the parents but failed to find any specific associations (i.e. 21-21) (Jacobs and Mayer, 1981).

Zellweger et al. (1966) were the first to systematically study satellite associations and Robertsonian translocation formation. They found that one parent in each of two families who had a child with a _de novo_ Robertsonian translocation had an increase in satellite associations over the control individuals. However, the parental origins of the _de novo_ rearrangements were unknown. More recently, Nikolis and Kekic (1986) studied 10 families having a child with _de novo_ 21q21q translocation Down syndrome. The parents of origin of the _de novo_ rearrangement had lower mean satellite associations for chromosome 21 and specifically for 21-21 associations than the control individuals. These findings may indicate that satellite associations of chromosomes 21 are not a causal factor in t(21q21q). Furthermore, these rearrangements may be isochromosomes of 21 in which decreased satellite

associations of chromosome 21 may be a factor in their formation.

D. Causes specific to isochromosome formation

Isochromosomes, as described by Darlington (1939; 1940), are chromosomes composed of identical arms. Because of this genetic identity, isochromosomes must arise from one chromosome. Therefore, the mechanisms by which isochromosomes arise are probably different from those which give rise to translocations.

There are three mechanisms postulated by which isochromosomes could form (Fig. 3): 1) misdivision of the centromere, 2) sister chromatid breakage with a U-type reunion, and 3) crossing over within a pericentric inversion. Very little is known about acrocentric isochromosomes since these mechanisms have been studied in nonacrocentric isochromosomes; mainly i(Xq) and isochromosomes that arise in leukemias and tumors [i.e. i(17q) and i(6p), respectively]. Isochromosomes can have varying morphologies which can provide important clues to the possible mechanisms that give rise to these rearrangements.

Misdivision, the first mechanism for isochromosome formation, refers to an erroneous transverse division of the centromere (Darlington, 1939; 1940). This mechanism has been used to explain the occurrence of symmetrical monocentric chromosomes. Misdivision of the centromere has been thought to give rise to i(Xq) (Priest et al., 1975; Hsu et al., 1978) and i(6p) in retinoblastoma tumor cells (Horsthemke et al.,

1989). However, this assumes that the byproducts [i(Xp) and i(6q), respectively] have been lost in subsequent cell divisions. Very few examples exist that show retention of both products: i(18p) and i(18q) in Edward syndrome (Müller et al., 1972; Larson et al., 1978) and i(17p) and i(17q) in two cells of a normal female (de la Chapelle, 1982). Although the retention of both products appears to be rare, the absence of one product may reflect cell viability or selection (de la Chapelle, 1982). In contrast, if the transverse division occurred in meiosis and proper segregation of the centromeres occurred, then only one product would be expected (fig. 3a).

An alternate mechanism which could explain both the occurrence of monocentric and dicentric isochromosomes is an isolocal break in sister chromatids with a U-type reunion (Fig. 3). First described by de la Chapelle et al. (1966), this mechanism has been used to explain the occurrence of dicentric isochromosomes (de la Chapelle and Stenstrand, 1974; Priest et al., 1975; Hsu et al., 1978). Recently, molecular evidence has shown that breakage and reunion of sister chromatids is the most likely mechanism responsible for forming dicentric Xq chromosomes (Harbinson et al., 1988; Phelan et al., 1988). Interestingly, together these molecular studies have shown an equal sex distribution in parental origin of the dic(Xq)s and since males have only one X chromosome, this lends support for "true" isochromosome formation (Harbinson et al., 1988; Phelan et al., 1988). Further support for sister chromatid exchange in isochromosome

Fig. 3. Three mechanisms of isochromosome formation. a. Misdivision (or transverse division) of the centromere leads to two monocentric products; one composed of the short arms and one of the long arms. b. Sister chromatid exchange and an erroneous U-type reunion leads to either a monocentric or dicentric product depending on the position of the exchange (centromere or short arm, respectively). Crossing over may occur distal to the exchange as indicated. c. A crossover in a pericentric inversion leads to a chromosome that appears to be an isochromosome but in fact is derived from the inverted chromosome and its normal homolog and is not a "true" isochromosome.

a.
S
AI
AII

b.
S
AI
AII

c.
recombination

formation comes from studies of homogeneously staining regions (HSRs) in the MeWo human melanoma cell lines (Holden et al., 1989). The amplified HSRs include a large inverted repeat of tandemly repeated sequences. The HSRs were involved in the formation of several isochromosomes. This suggests that inverted repeats could facilitate sister chromatid exchanges leading to isochromosome formation. Tandemly repeated sequences are found in the pericentromeric and nucleolus organizer regions of the human acrocentric chromosomes.

A third mechanism for isochromosome formation involves crossing over within a pericentric inversion. Originally proposed by Nusbacker and Hirschhorn (1968), the morphology of the resulting isochromosome would depend on the area of the chromosome involved in the inversion. This mechanism has been used to explain two cases of i(Xq) (Priest et al., 1975; Hsu et al., 1978). However, this mechanism is the least attractive because of its complexity. Furthermore, the most frequent pericentric inversion involves chromosome 9; inv(9) constitutes 40% of all pericentric inversions (Therman, 1980) yet i(9p) and i(9q) are rarely reported (Van Dyke, 1988) and inversions in these parents have not been documented. Recently, electron microscopy (EM) of a pericentric inversion of chromosome 21 was studied (Gabriel-Robez et al., 1988). The chromosome 21 bivalent could be identified by its kinetochore nonalignment. Loop formation was not observed. This was speculated to result from the small size of chromosome 21 since inversions of larger chromosomes (i.e.

chromosome 1) have been observed to have inversion loops on EM study (Gabriel-Robez et al., 1988). The nonalignment of the kinetochores which leads to pairing of nonhomologous segments would minimize the opportunity for proper crossing over and therefore limit the formation of duplications and deficiencies through 'aneusomie de recombinaison' (Gabriel-Robez et al., 1988). Thus, isochromosome 21 formation would not be expected from carriers of pericentric inversions of chromosome 21. A final note about pericentric inversions in isochromosome formation comes from Schmutz and Pinno (1986). They reported an i(18q) in a child born to a mother with a pericentric inversion 18 [inv(18)(p11.3q11.2)]. As they point out, a crossover event in an inversion loop would lead to a chromosome similar to an isochromosome in morphology but the arms would be derived from the inv(18) and its homolog and therefore would not be genetically identical, thus not a "true" isochromosome.

V. Consequences of acrocentric exchanges: Nonrandom participation of acrocentric chromosomes in rearrangements

A. Satellite DNA

Satellite DNA, found in the short arms of the acrocentric chromosomes, was shown to be present in dicentric Robertsonian translocations although always less than the amount in the "normal" homologs (Gosden et al., 1981). No satellite DNA could be detected from the missing short arms in the monocentric Robertsonian translocations tested (Gosden et al., 1981). The breakpoints in dicentric Robertsonian

translocations are most likely in the satellite DNA of the short arms causing a reduction, but rarely a complete loss, of this DNA (Gosden et al., 1981).

Quantities of satellite III DNA, by in situ hybridization studies, were found to be highest in chromosomes 15 and 22 and lowest in chromosomes 13, 14, and 21 (Gosden et al., 1979; 1981). Gosden et al. (1979) suggested that the nonrandom participation of the acrocentrics in Robertsonian translocations was related to the amount of satellite III DNA found on the different chromosomes. Chromosomes with smaller amounts of satellite DNA are "tolerated" better in Robertsonian translocations (i.e. chromosomes 13 and 14). The chromosomes that contain the largest amounts of satellite DNA (i.e. 15 and 22) were rarely found in Robertsonian translocations. It is possible that a substantial loss of this DNA, through Robertsonian translocation formation or other mechanisms, would be deleterious. Furthermore, the retention of satellite DNA in these rearrangements may be necessary for cell survival and this may explain why the majority of Robertsonian translocations are dicentric.

Chromosomes 15 and 22, which contain the highest amount of satellite III DNA, have been reported to participate in satellite associations more frequently than the other acrocentric chromosomes (Gosden et al., 1978; Ray and Pearson, 1979). Thus, the amount of satellite III DNA may be related to satellite associations and if so, loss of this DNA may alter chromosomal participation in satellite associations.

Hansson (1975) found that the presence of Robertsonian translocations caused a higher frequency of satellite associations among the remaining homologs. However, Zankl and Zang (1978) did not find an alteration in satellite association participation after the loss of D and G chromosomes in meningiomas. It is assumed that satellite associations are necessary for nucleolus formation and an alteration in satellite associations through the formation of certain Robertsonian translocations may be deleterious and could explain the apparent nonrandomness observed in Robertsonian translocations.

B. Nucleolus organizer region

Although individual differences exist in the amount of silver-staining on the acrocentric chromosomes (Bloom and Goodpasture, 1976), some trends have been established. In one study, the frequency of silver-positive NORs were lowest in chromosomes 14 and 22 and equally high in 13, 15 and 21 (Mikelsaar et al., 1977) while in another study, chromosome 21 had the largest amount of silver stain and chromosome 15 had the least (Zakharov et al., 1982). Clearly, NOR activity alone does not account for the nonrandom participation of acrocentric chromosomes in rearrangements.

It is possible that the amount of NOR material lost (or remaining) dictates the viability of a rearrangement. Compensation of NOR activity has been studied. Compensation refers to a mechanism by which other acrocentric chromosomes not involved in the centric fusion increase their activity to

make up for the absent NORs. Zankl and Hahmann (1978) studied the cells of a carrier of a de novo t(13q13q). They found that the loss of NORs through the formation of the rearrangement was not compensated for by the other acrocentric chromosomes. However, the satellite associations remained consistent in the proband as compared to her parents because of an increase in chromosome 22 satellite associations. Other studies of compensation have had mixed results. Gosden et al. (1979) found evidence of compensation in a proband with a de novo t(13q14q) through the activation of a chromosome 21 shown to be inactive in the mother. However, Jotterand-Bellomo and Van Melle (1981) did not find evidence of compensation in four carriers of Robertsonian translocations studied. Additionally, in a study of a family with a t(13q14q), no evidence for compensation was found (Nikolis et al., 1981) whereas a compensatory mechanism was found in probands with t(21q21q) who had Down syndrome (Nikolis and Kekic, 1988). Two explanations of the results can be offered: 1) A compensatory mechanism exists for some translocations (i.e. 21q21q) but not others (i.e. 13q14q) or 2) the data were not corrected for the fact that the carriers of the t(13q14q) were "balanced" and the carriers of the t(21q21q) had Down syndrome and therefore had an additional chromosome 21 that could increase the total NOR score. Furthermore, the t(13q14q)s could potentially be losing two active NORs whereas the t(21q21q)s may actually be i(21q)s thus potentially losing only one active NOR. Finally, "reverse" compensation has not

been documented in probands with trisomy 21 (Wegner et al., 1980).

C. Dicentric formation

The majority of Robertsonian translocations examined have been shown to be dicentric (Niebuhr, 1972; Daniel and Lam-Po-Tang, 1976; Mattei et al., 1979). Dicentric chromosomes can be unstable because of the formation of anaphase bridges and subsequent breakage as observed in Bloom syndrome (Therman, 1986). The stability of dicentric Robertsonian translocations has been studied extensively. The mechanisms by which stability is achieved have been hypothesized: 1) The close proximity of the centromeres causes them to act in unison (Daniel and Lam-Po-Tang, 1976; Lau and Hsu, 1977); 2) centromere suppression, dominance or inactivation (Daniel and Lam-Po-Tang, 1976; Therman et al., 1986); 3) deletion of one centromere (Vianna-Morgante and Rosenberg, 1986); and 4) disorganization of the kinetochore in the inactive centromere (Wandall, 1989).

The first hypothesis for dicentric stability was investigated using Cd-banding of L strain mouse cells (Lau and Hsu, 1977). Cd-banding stains only the active centromeres (Eiberg, 1974). The mouse cells display a variable number of Robertsonian-type fusions. Cd-banding revealed that in the rearrangements where the centromeres were very close, with no heterochromatin between them, two Cd-bands were present. Even in a few cases where there was some heterochromatin between the centromeres, both centromeres stained Cd-positive. These

findings suggest that dicentric Robertsonian translocations may retain two functional centromeres (Lau and Hsu, 1977).

The second hypothesis of dicentric stability involves the inactivation or suppression of one centromere. This is evident in the rearrangement by the presence of one primary constriction but two C-bands (Therman et al., 1986) and in some cases a single Cd-band (Daniel, 1979). [C-banding stains the large heterochromatic regions of chromosomes 1, 9, 16 and Y and the centromeres of all chromosomes (Benn and Perle, 1986)].

Unfortunately, chromosomal stains give little information about the actual process by which one centromere is inactivated. In a case of a dic(13;20), 60% of the cells presented the translocation as a dicentric with the centromere 13 active (demonstrated by a primary constriction) and the centromere 20 suppressed (demonstrated by a positive C-band). In 40% of the cells, the chromosome was monocentric with an active centromere 20 and a deleted centromere 13 (Vianna-Morgante and Rosenberg, 1986). They postulated that in the cells in which the dicentric chromosome was unstable, anaphase bridge formation and breakage contributed to the cells that had the deleted centromere 13. These cells appeared to be stable since no cells were observed that had this chromosome completely absent. This rare example may be the exception however and not the rule.

For the last hypothesis of dicentric stability, a tdic(21;21)(q22;q22) was examined (Wandall, 1989). This

dicentric chromosome occasionally demonstrated two primary constrictions at the centromeres(22.5%) but Cd-banding showed only one active centromere. Centromere-specific autoantibodies reacted weakly to one centromere (inactive) and strongly with the other (active). The weak antibody reaction indicated that a kinetochore would probably not develop. Electron microscopy confirmed that kinetochore development was only at one centromere. However, a tdic(5;13)(p12;p12) had kinetochore development at both centromeres. Thus, kinetochore development may be centromere (or rearrangement) specific.

Finally, molecular probes for chromosome specific centromeres are being developed (Willard, 1985). These probes may allow for the identification of the active centromere(s) in Robertsonian translocations. The retention of certain active centromeres may lead to the apparent nonrandom participation of acrocentric chromosomes in Robertsonian translocations. Furthermore, particular functional centromeres may lead to more viable segregation outcomes that are ascertained in abortuses or liveborns [i.e. t(13q21q) with a functional centromere 21].

D. Summary of the nonrandomness of Robertsonian translocations

In summary, the nonrandom participation of acrocentric chromosomes remains essentially unresolved for a number of reasons: 1) Ascertainment alone cannot explain the observation of nonrandomness. If more t(14q21q) are

ascertained because of the Down syndrome phenotype, this does not explain the rarity of t(13q21q) and t(15q21q) among this group (Therman et al., 1989). Likewise, ascertainment alone does not explain the high frequency of t(21q21q) and it is possible that some of these are isochromosomes and these rearrangements could arise through more than one mechanism. 2) Satellite association is probably the major mechanism by which the acrocentric chromosomes occupy close physical proximities. However, the evidence is weak that certain satellite associations give rise to certain Robertsonian translocations (Gosden et al., 1978; Ray and Pearson, 1979; Therman et al., 1989). 3) Staining properties of the acrocentric short arms and NORs have shed little light on this nonrandomness since individual heteromorphisms in QFQ and NOR staining exists (Bloom and Goodpasture, 1976; Mikelsaar et al., 1977; Morton et al., 1981; Zakharov et al., 1982). 4) The molecular variation in the pericentromeric regions, especially of chromosomes 13, 14 and 21 may provide the most information to explain the high frequency to t(13q14q) and t(14q21q) (Choo et al., 1988; 1989). Additionally, as more is learned about the DNA content of the pericentromeric regions, information may be gained about the deleterious effects of losing satellite DNA which may contribute to the observed nonrandomness (Gosden et al., 1978). 5) Finally, the mechanisms involved in dicentric stability may give rise to the apparent nonrandomness by allowing cells carrying specific translocations to survive by avoiding anaphase bridge

formation and chromosome breakage.

VI. Studies of the parental origin of de novo acrocentric rearrangements

A. Chromosomal staining variants

The origin of the extra chromosome in Down syndrome has been identified through the use of quinacrine fluorescence variants of chromosome 21 (Juberg and Mowrey, 1983); quinacrine and NOR variants (Mikkelsen et al., 1980; Jackson-Cook et al., 1985); and with the addition of fluorescent R-banding (Verma et al., 1986). The parental origin of nondisjunction could be determined in about 50% of families studied using only the quinacrine variants and an additional 30% could be determined by combining the ammoniacal silver stain with quinacrine fluorescence (Jackson-Cook et al., 1985). The consensus among the studies is that maternal nondisjunction accounts for 80% and paternal nondisjunction accounts for 20% of cases of trisomy 21 (Juberg and Mowrey, 1983). These staining variants have also been used to study; 1) the parental origin of the extra chromosome 13 in Patau syndrome (Hara and Sasaki, 1975; Ishikiriyama and Miikawa, 1984); 2) paternity (Olsen et al., 1986); 3) the parental origin of the chromosome in trisomic spontaneous abortuses (Hassold et al., 1987); and 4) the parental origins of de novo reciprocal and Robertsonian translocations (Tables 7 and 8). Since the staining heteromorphisms are often lost in the formation of the Robertsonian rearrangement, the parental origins must be assigned for the free-lying chromosomes and

the parental origin of the de novo rearrangement is thus determined by exclusion (Chamberlin and Magenis, 1980).

Thirty-four cases of de novo acrocentric rearrangements in which the parental origin was known are summarized in Tables 7 and 8. These studies of de novo Robertsonian translocations are divided between those which occurred between nonhomologous chromosomes (Table 7) and those between homologs (Table 8). The number of maternally and paternally derived de novo rearrangements are similar when the Tables 7 and 8 are combined (20 maternal, 14 paternal). However, there is a significant excess of maternally derived de novo Robertsonian translocations (8 maternal, 1 paternal) among those involving non-homologous pairs (X^2_1=5.44 $p<0.025$) (Table 7). For these "true" Robertsonian translocations, the parental origin closely resembles the parental origin of nondisjunction in trisomy 21 (80% maternal). The parental origins of the homologous rearrangements are equally distributed between maternal (12) and paternal (13) (Table 8). The differences in parental origins between "true" Robertsonian translocations and rearrangements between homologous chromosomes indicate that their etiologies may differ. It is possible that if homologous rearrangements could be divided between rob(21q21q) and i(21q), a pattern of parental origins would emerge.

Chromosomal variants have been identified in some parents who have a child with a de novo acrocentric rearrangement (Table 9). In two families, the parent of origin of the

Table 7. Parental origin of de novo Robertsonian translocations between nonhomologous chromosomes

Robertsonian translocation	Origin Mat	Origin Pat	Staining Variant	Source
t(13q14q)	1	0	Ag-NOR/DAPI	Gosden et al.,1979
t(13q14q)	1	1	QFQ	Chamberlin and Magenis, 1980
t(13q21q)	1	0	QFQ	Pérez-Castillo and Abrisqueta, 1978
t(14q15q)	1	0	QFQ	Jacobs et al., 1974
t(14q21q)	1	0	QFQ	Robinson, 1973
t(14q21q)	2	0	QFQ	Chamberlin and Magenis, 1980
t(14q21q)	1	0	QFQ/Ag-NOR	Mikkelsen et al., 1980
Total	8	1		

Table 8. Parental origin of de novo rearrangements between homologous chromosomes

Rearrangement	Origin Mat	Origin Pat	Staining Variant	Source
rea(21q21q)[a]	1	0	QFQ	Schmidt et al., 1975
rea(21q21q)	0	1	QFQ	Hara and Sasaki, 1975
rea(21q21q)	0	1	QFQ	Magenis et al., 1977
rea(21q21q)	1	0	QFQ	Jacobs et al., 1978
rea(21q21q)	3	1	QFQ	Mattei et al., 1979
rea(21q21q)	4	1	QFQ	Chamberlin and Magenis, 1980
rea(21q21q)	1	1	QFQ/Ag-NOR	Mikkelsen et al., 1980
rea(21q21q)	1	3	QFQ/Ag-NOR	Nikolis and Kekic, 1986
i(21q)	1	0	RFLP	Créau-Goldberg et al., 1987
i(21q)	4	0	QFQ/RFLP	Grasso et al., 1989
rea(21q21q)	0	2	QFQ/RFLP	Grasso et al., 1989
rea(13q13q)	1	0	QFQ/RFA	Kajii et al.,1976
rea(13q13q)	0	3	QFQ	Chamberlin and Magenis, 1980
t(21;21)(q21;p13)	0	1	RFA	Verma et al., 1977
t(21;21)(q22;q22)	0	1	QFQ/Ag-NOR	Pfeiffer and Loidl, 1982
Total	17	15		

[a] rea is used to denote those rearrangements in which Robertsonian translocations could not be distinquished from isochromosomes.

de novo rearrangement was not the parent who possessed a chromosome variant (Jacobs et al., 1974; Verma et al., 1977). In another family, the mother carried a 21p- and was the origin of her son's de novo t(21q21q). Pérez-Castillo and Abrisqueta (1978) described a family in which both parents carried a chromosome variant and thus, is uninformative for chromosome variant involvement in the formation of the de novo rearrangement. Lastly, Jackson-Cook et al. (1988) described an apparently non-mosaic mother who had two dNOR variant chromosomes and had two children with rea(21q21q) from different males. The involvement of the variant chromosomes could not be established since the parental origins of the de novo rearrangements were not determined.

B. Restriction fragment length polymorphism analysis

Restriction fragment length polymorphisms (RFLPs) have been used by several investigators to assign the parental origin and meiotic stage of the nondisjunctional error resulting in trisomy 21 (Davies et al., 1984; Stewart et al., 1985; Hamers et al., 1987; Stewart et al., 1988; Dagna Bricarelli et al., 1988; Rudd et al., 1988; Galt et al., 1989). Over 50 DNA markers have been identified for chromosome 21 (Stewart et al., 1988). Twenty-four of these markers have been mapped through recombination studies (Petersen et al., 1989). Molecular probes have been identified which span the length of chromosome 21q, but there is an absence of useful markers for 21p (Tanzi et al., 1988; Warren et al., 1989; Petersen et al., 1989). The DNA markers

Table 9. Summary of chromosome variants in parents of children with a de novo acrocentric rearrangement

Variant	Parent	rea	P.O.	Source
15ps+	father	t(14q15q)	mother	Jacobs et al., 1974
1qh+	mother	t(21;21)(q21;p13)	father	Verma et al., 1977
21p-	mother	t(21q21q)	mother	Jacobs et al., 1978
Yq+,15ps+ 22ps+	father mother	t(13q21q)	mother	Pérez-Castillo and Abrisquetta, 1978
14,22 dNOR(+)	mother	rea(21q21q)	unknown	Jackson-Cook et al., 1988

become helpful tools for studying nondisjunction especially in cases where the cytogenetic markers are uninformative or in question (Stewart et al., 1988; Millington-Ward and Pearson, 1989). By utilizing a sufficient number of DNA markers spanning the length of the chromosome, it is possible to assign the parental origin of nondisjunction in virtually all cases of trisomy 21 (Stewart et al., 1988; Chakravarti, 1989).

To date, DNA markers have been used to determine the parental origins of acrocentric rearrangements in three studies (Créau-Goldberg et al., 1987; Priest et al., 1988; Grasso et al., 1989) (Table 8). In two cases the rea(21q21q) was determined to be maternally derived, one from a mosaic mother (Priest et al., 1988) and the other a _de novo_ event (Créau-Goldberg et al., 1987). In the largest study to date, four of six cases of rea(21q21q) were determined to be maternally derived isochromosomes by RFLP analysis (Grasso et al., 1989). In the future, molecular markers will continue to be useful in distinguishing isochromosomes from homologous Robertsonian translocations, observing recombination between translocations and normal homologs, and identifying the molecular sequences involved in Robertsonian exchanges.

MATERIALS AND METHODS

I. Ascertainment of the study subjects

The cytogenetic analyses in this study were completed on a total of 181 individuals; 26 children who have a *de novo* rearrangement of the acrocentric chromosomes, their parents (52), and available siblings (18), 25 carriers of familial Robertsonian translocations, 50 control individuals and 10 other individuals including children of familial carriers (7) and other family members (3). A list of all individuals karyotyped at the Medical College of Virginia (MCV) with a Robertsonian translocation was obtained from the service laboratories in the Department of Human Genetics (Table 10). Carriers of Robertsonian translocations were determined to be *de novo* or familial by previously karyotyping other family members or by reviewing family histories that have been collected in the Department of Human Genetics. A total of 103 individuals were found to carry a Robertsonian translocation between the years of 1965 and 1989. Letters briefly explaining the study were sent to those families with complete addresses. Of the 56 letters sent, 14 (25%) expressed a desire to participate in the study, 11 (19.6%) did not want to participate and 31 (55.4%) of the letters were

Table 10. Cytogenetic findings among patients with Robertsonian translocations karyotyped at the Medical College of Virginia from 1965-1989.

	D/D		D/G		G/G	
	D/D	2	D/G	2	G/G	1
	13/13	1	13/22	1	21/21	19
	13/14	36	14/21	6	21/22	6
	14/15	10	14/22	2		
	15/15	1	15/21	15		
			15/22	1		
Total		50		27		26

either undeliverable or received no reply. Therefore, a total of six families who had a child with a de novo Robertsonian translocation and eight carriers of familial Robertsonian translocations were ascertained from the cytogenetic records at MCV. Four additional families (1 de novo and 3 familial carriers) were ascertained during this investigation through the MCV Genetic Counseling Clinic. In addition, 37 cytogenetic centers were contacted for the ascertainment of additional families and 18 of these centers agreed to participate. Blood samples or prepared slides from 16 families were received from eight different centers. Eight of these families were unanalyzable because of poor slide preparations (6) or incomplete families (2). Therefore, a total of five individuals with de novo Robertsonian translocations and their parents and three familial carriers and their spouses, were ascertained from other genetic centers. Letters were sent to 166 Down syndrome parents organizations across the United States. From this source, 14 families who have a child with a de novo rearrangement and 11 carriers of familial Robertsonian translocations participated in this investigation. A summary of the ascertainment is given in Table 11.

Fourteen control couples were ascertained from a population-based twin panel established by the Department of Human Genetics at MCV. The 28 individuals were the parents of twins who were participating in studies through the Department of Human Genetics. Pedigrees from all 14 couples

Table 11. Summary of ascertainment of study subjects

	Number of Families		
	de novo	Familial	Source
	7	11	MCV
	5	3	other genetic centers[a]
	14	11	Down syndrome parents organizations
Total	26	25	

[a] A. Brothman, Eastern Virginia Medical School, Norfolk, VA
S. Black and J. Schulman, Genetics and IVF Institute, Fairfax, VA
T. Hassold and K. May, Emory University, Atlanta, GA
P. Howard-Peebles, Genetics and IVF Institute, Fairfax, VA
E. Magenis, Oregon Health Sciences University,Portland,OR
R. Neu, Vivigen Inc., Santa Fe, NM
H. Wyandt, Boston University, MA

were collected to assess their reproductive histories. One couple was found to be a multiple aborter. An additional 22 control individuals were ascertained through their spouses who were carriers of familial Robertsonian translocations.

The molecular genetic analyses were undertaken for all parents and children who had a de novo rearrangement resulting in a trisomy (22/26 families). Eight families could not be completed because of a lack of recoverable DNA from their samples. Therefore, the molecular genetic analyses were completed on a total of 14 families; 57 individuals comprised of 28 chromosomally normal parents, 13 individuals who had an acrocentric chromosomal rearrangement leading to a trisomy, one individual who had a de novo Robertsonian translocation and trisomy 21, and 15 chromosomally normal siblings.

II. Establishment of lymphocyte cultures

Three to ten milliliters of heparinized venous blood for chromosome analyses were obtained from the child with the de novo acrocentric rearrangement, their parents and available siblings. Ten milliliters of heparinized blood was obtained from the individuals who carried a familial Robertsonian translocation, their spouses, and the control individuals. At the time of the blood drawing, consent forms were signed (appendix), the study was further explained and questions from the study participants were answered. For those individuals who lived outside the state of Virginia, extensive counseling was conducted over the phone. Blood was drawn by their local physicians or genetic counseling clinics and was shipped at

room temperature by an overnight express carrier.

The lymphocyte cultures were established according to our modification of the method by Moorhead (1960). The blood was transferred to a sterile 15ml centrifuge tube and centrifuged at a speed of 900 rpm for 8 minutes. The buffy coat was removed sterilely with a 1ml pipet and 0.05ml was placed into each of two $25cm^2$ plastic culture flasks containing 10ml media (Grand Island Biologic Company [GIBCO] McCoys 5A media Spinner modified with 15% FBS) and 0.5ml PHA (Wellcome). The cultures were mixed gently by shaking and allowed to incubate upright with the caps loosened for 72 hours at 37°C in ambient air and 5% CO_2. The following modifications were established for blood received by overnight express mail; 1) samples less than 3ml were cultured by adding 0.5ml of whole blood, and 2) samples were cultured for 96 hours with an increase in mitotic index achieved.

III. Cell harvest and slide preparation

After 71.25 hours of incubation, 0.1ml of colcemid (GIBCO 10μg/ml stock solution) was added to each culture, mixed gently and returned to the incubator for 45 minutes. The contents of each flask were transferred to a 15ml centrifuge tube. Each flask was rinsed with 2ml Hank's balanced salt solution without calcium or magnesium (GIBCO #310-4170AJ) and added to the respective tube. The cell suspensions were centrifuged at 800 rpm for 8 minutes. The supernatants were removed by aspiration and the cell pellets were resuspended by vortexing in 7ml of hypotonic solution [0.075M KCl (Fisher

Scientific)] at 37°C and incubated at room temperature for 20 minutes. At the end of the incubation, 4 drops of Carnoy's fixative (3:1 absolute methanol, Baker: glacial acetic acid, Baker) were added, the cells were vortexed and centrifuged at 800 rpm for 8 minutes. Following centrifugation and aspiration of the supernatant, the pellet was resuspended in 6ml Carnoy's fixative and incubated at room temperature for 10 minutes. The pellet was collected by centrifugation, resuspended in 6ml Carnoy's fixative, and centrifuged omitting the incubation time. This step was repeated for a total of three changes before the slides were prepared. Cell suspensions in fixative were dropped onto ice cold chromic acid cleaned slides using a siliconized 9inch pasteur pipet, tapped ten times, and heated on a hot plate at 60°C for 15 seconds. The slides were labelled and stored for at least 2 weeks to permit aging before staining with ammoniacal silver and quinacrine mustard. The slides were used within 24 hours for C-banding.

Modifications of the lymphocyte protocol were as follows for amniocytes and tissue cultures. Flasks were incubated at 37°C in ambient air and 5% CO_2 and monitored daily at the microscope in order to harvest at the optimal time of active cell growth. Colcemid (10μg/ml stock solution) was added in the amounts of 0.1ml for 3 hours and 0.3ml for 4 hours to each 25cm^2 flask containing amniocytes and tissue cultures, respectively. After the incubation period at 37°C, the media from each flask was transferred to the appropriate centrifuge

tube, 2ml of trypsin-EDTA (GIBCO) was added to each flask, washed over the attached cells for 2 minutes and then added to the appropriate tube. This was repeated and the flasks were then placed in the 37°C incubator for 3-5 minutes to lift the cells. The flasks were agitated gently to dislodge the cells and checked at the inverted microscope (Zeiss) for completion. Hank's balanced salt solution (2ml) was added to each flask to wash out the lifted cells. Cell pellets were collected by centrifugation at 1000 rpm for 7 minutes. Following aspiration of the supernatant, the cell pellet was vortexed and 5ml of 37°C hypotonic solutions of 0.7% sodium citrate (Mallinckrodt) for 2 minutes or 0.075M KCl for 7 minutes were added to amniocyte and tissue cultures, respectively. Four drops of Carnoy's fixative were added at the completion of the hypotonic incubation and the cell pellet was collected by centrifugation. Following aspiration of the supernatant and resuspension of the cell pellet, 7ml of cold fixative was added to each tube and incubated for 30 minutes at 4°C. The cells were washed twice with cold fixative. The cell suspensions were dropped onto cold, wet slides and heated in the steam of a beaker of boiling water for 5-10 seconds.

IV. Chromosome staining

Chromosomes were stained simultaneously with ammoniacal silver and quinacrine mustard dihydrochloride according to our modifications of the methods by Bloom and Goodpasture (1976) and Caspersson et al. (1971), respectively (Jackson-Cook, 1985) for the identification of the acrocentric

chromosomes and heteromorphism scoring. Aged slides were soaked for 10 minutes each in a series of ethanol dilutions; 100%, 90%, 70% and 30%. Slides were soaked for 10 minutes in MacIlvaine's buffer (pH 5.4) (0.1M citric acid, Baker and 0.2M Na_2HPO_4, Mallinckrodt). Each slide was rinsed 10 times in Millipore water and blotted dry with filter paper. Three drops of a 50% silver nitrate (Baker) solution in Millipore water (SI) was placed on the slide, covered with a coverslip, and placed on a 85°C hot plate for 30 seconds at which time, the solution boiled. The coverslip was washed off with Millipore water and blotted dry with filter paper. One drop of a chilled SII solution (2g $AgNO_3$, Baker in 2.5ml Millipore water and 3.75ml NH_4OH, Fisher Scientific) and one drop of 3% formalin, pH 7.2 (10% formalin stock solution, Baxter) were added near the frosted end of the slide and covered with a coverslip. The reaction was monitored in the center of the slide using a phase microscope (Zeiss, 25X objective). The stalk regions of some of the acrocentric chromosomes turned black and the chromosomes turned a golden brown. After this color change, the coverslip was immediately rinsed off with Millipore water and blotted dry with filter paper. The slides were then stained in a 0.005% solution of quinacrine mustard dihydrochloride (Sigma) in MacIlvaine's buffer (pH 5.4) for 26 minutes. Each slide was rinsed 10 times and soaked for 10 minutes in MacIlvaine's buffer (pH 5.4). After allowing the slides to air dry at room temperature overnight, the slides were coded in a random order by a co-worker. The slides were

viewed by mounting them with 2 drops of MacIlvaine's buffer (pH 4.3) and a coverslip.

Fresh slides from subjects with de novo acrocentric rearrangements were C-banded for centromeric determination according to Benn and Perle (1986). Slides were treated in the following manner; 0.2M HCl (2N, Sigma) for 30 minutes at room temperature, rinsed in Millipore water two times, 0.07M $Ba(OH)_2$ (0.3N, Sigma) for 10 minutes at 37°C, rinsed in Millipore water three times, 2X SSC (0.03M sodium citrate and 0.03M NaCl, Fisher Scientific) at 65°C for 2 hours, rinsed in Millipore water and stained for 2 hours in 10% giemsa (Gurr) in phosphate buffer pH 6.8 (0.025M KH_2PO_4, Fisher Scientific). Slides were viewed with a light microscope under oil emersion (Leitz, 63X objective) (Fig. 4).

V. Chromosome analysis: Scoring QFQ and NOR heteromorphisms and satellite associations

Ten mid-metaphase chromosome spreads were examined for QFQ and NOR heteromorphisms and satellite associations in each individual. QFQ and NOR heteromorphisms were scored simultaneously using ultra-violet and visible light sources. The centromere (chromosomes 13 and 22 only), short arm and satellite regions of the acrocentric chromosomes were scored according to the QM staining intensities as established at the International System for Human Cytogenetic Nomenclature Paris Conference (1972) (Fig. 5). The NOR heteromorphisms were scored according to the method by Markovic (1978) as modified by Morton (1983) (Fig. 5).

Fig. 4. C-banded preparation. The centromeres of all chromosomes and the heteromorphic regions of chromosomes 1, 9, 16 and Y stain darkly. For example, the rearrangement from the proband of family 1 was dicentric as indicated by the arrow.

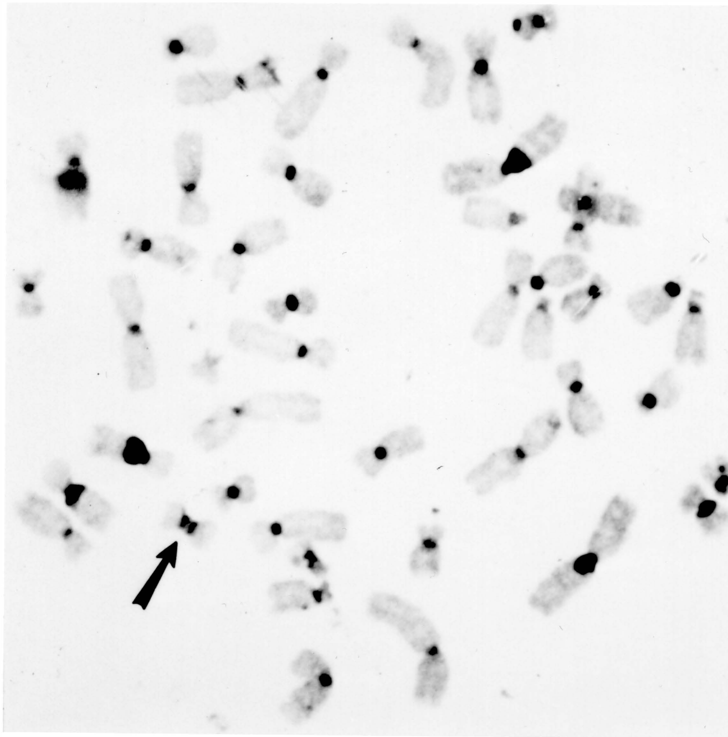
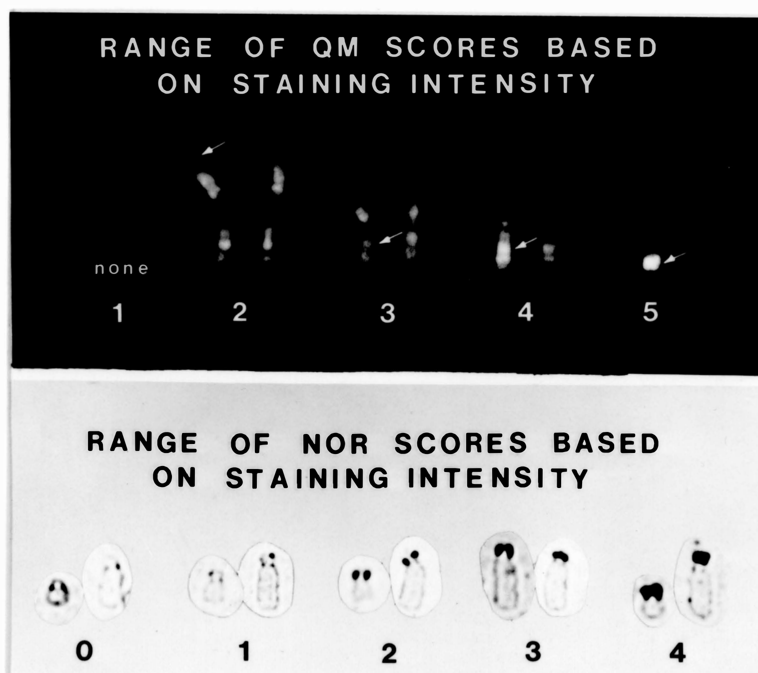


Fig. 5. Standards for scoring QFQ (top) and NOR (bottom) heteromorphisms. a. The QFQ heteromorphisms were scored on a scale from one to five. A score of one for no fluorescence; two for pale fluorescence as distal 1p; three for medium fluorescence; four for intense fluorescence as distal 13q; and five for brilliant fluorescence as distal Yq. b. The NOR heteromorphisms were scored on a scale from zero to four. A score of zero for no stain; one for one dot of stain; two for two small dots; three for two large dots or a continuous area of stain; and four for a large continuous area of stain. The two areas of stain in a double NOR variant were scored separately then added together as one score for the chromosome. (Courtesy of Dr. Jackson-Cook)


RANGE OF QM SCORES BASED
ON STAINING INTENSITY
none
1
2
3
4
5
RANGE OF NOR SCORES BASED
ON STAINING INTENSITY
0
1
2
3
4

Satellite associations were scored according to the modifications of Ing (1975) of the methods of Cohen and Shaw (1967). Acrocentric chromosomes were considered to be in association if their satellites were no further apart than the length of a D group chromosome in the spread (Fig. 6).

VI. Cytogenetic determination of parental origins of de novo acrocentric rearrangements

Parental origins of the de novo rearrangements were determined using QFQ and NOR heteromorphisms and chromosomal morphologies from microscopic evaluations, photographs and comparisons of mean QFQ scores and mean NOR scores for each acrocentric implicated in the rearrangement. Comparisons of the heteromorphisms were made between the parents and offspring. Since the heteromorphic regions were usually lost during the formation of the rearrangement, the parental origin was determined for the normal free-lying acrocentric homologs. Once established, the parental origin of the rearrangement was determined by exclusion (Fig. 7).

VII. Human genomic DNA extraction

Approximately 20ml of peripheral blood in citrate, EDTA or heparin was obtained from the child with the de novo rearrangement, their parents and available siblings. The DNA was extracted according to the protocol of Spence et al. (1987). The blood was transferred to a 50ml conical tube and centrifuged for 15 minutes at 2500 rpm. Plasma was removed with a sterile pipet and discarded. Lysis solution was added to the buffy coat and red blood cells to a final volume of

Fig. 6. Satellite associations of the acrocentric chromosomes. Chromosomes were considered to be in association if their satellites were no further apart than the length of a D group chromosome in that spread. Satellite associations are indicated by the arrows.

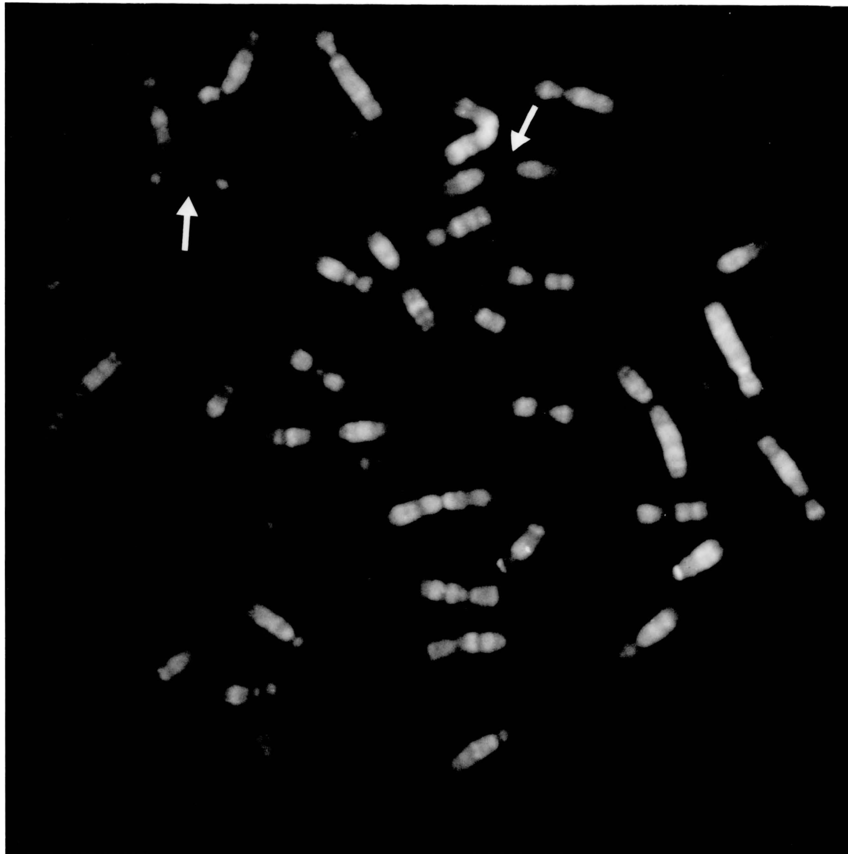
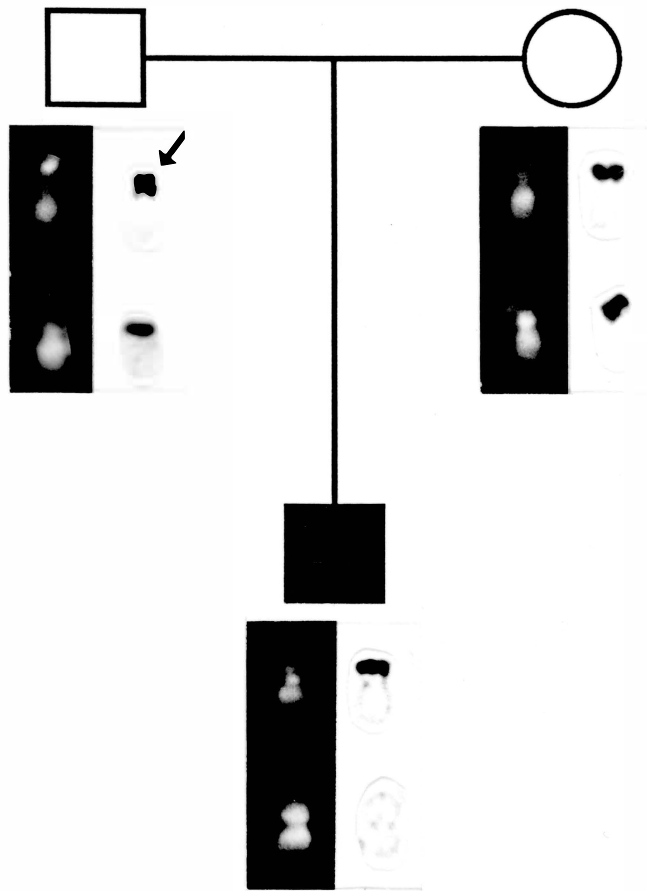


Fig. 7. Determination of parental origins of de novo acrocentric rearrangements using QFQ and NOR heteromorphisms. The parental origin was determined for the normal free-lying homolog. Once established, the origin of the rearrangement was determined by exclusion. The QFQ and NOR heteromorphisms are shown for family 4 (left and right, respectively). The proband inherited the maternal chromosome 21 with a brightly fluorescent short arm [var(21)(p11,QFQ5)mat] (lower of maternal complement) as the free-lying chromosome (upper of proband complement); indicating that the de novo (21q21q) was inherited from the father. The father has a dNOR variant on chromosome 21 as indicated by the arrow.



50ml. (Lysis solution: 0.32M sucrose, Bethesda Research Laboratories (BRL); 10mM TrisHCl pH 7.5, Sigma; 5mM $MgCl_2$, Fisher Scientific; and 1% Triton X-100, Sigma). The tubes were inverted for gentle mixing and placed on ice for 30 minutes. After centrifugation for 15 minutes at 2500 rpm, the supernatant was decanted, the cell pellet was resuspended in 25ml of lysis solution and centrifuged for 15 minutes at 2500 rpm. The nuclear pellet was resuspended in 4.5ml of a 10mM TrisHCl solution pH 7.4 containing 10mM NaCl(Fisher Scientific), 10mM Na_2EDTA (Sigma), 250μl of 20% sodium dodecyl sulfate (SDS) (BRL) and 200μl proteinase K (10mg/ml, Boehringer Mannheim) and incubated overnight at 37°C. After the incubation, 5ml phenol (Boehringer Mannheim) : chloroform (Baxter) : isoamyl alcohol (Fisher Scientific) (50:48:2) was added and rocked gently at room temperature for 15 minutes. The mixture was centrifuged for 15 minutes at 1000 rpm to separate the phases. The phenol (lower phase) was discarded and the extraction was repeated. Five milliliters chloroform: isoamyl alcohol (98:2) was mixed and centrifuged as described above to separate phases. A wide tip 25ml pipet was used to collect the upper aqueous phase. The volume was noted and 1/10X volume of 3.0M sodium acetate pH 4.8 (Fisher Scientific) and 2.2X volume of 100% ethanol were added to precipitate the DNA. The white clump of DNA was removed and washed in 70% ethanol. The DNA was dried in a Speedvac Concentrator (Savant) for 20 minutes. One milliliter of TE (10mM TrisHCl pH 7.4, 1mM Na_2EDTA pH 8.0) was added and the DNA allowed to

go into solution at 37°C for about 1 hour. The DNA was stored at 4°C. The optical density of the sample was read at UV 260nm using a Shimadzu spectrophotometer and the concentration in μg/ml was recorded.

VIII. Restriction enzyme digestion of human genomic DNA

For each probe-hybridization reaction, 7.5μg of total human genomic DNA was digested with the appropriate restriction enzyme under the temperature and buffer conditions specific for each enzyme (Table 12). In general, a total volume of 60μl was used for each individual digestion. The volume was composed of the DNA sample, 10X buffer (BRL), 5U enzyme/μg DNA and sterile water. After a 2 hour to overnight digestion, 10X loading buffer (0.15% Bromophenol Blue; Sigma, in 50% glycerol; Fisher Scientific) was added to each sample. The samples were loaded into the gel wells.

IX. DNA fractionation and Southern blot hybridization

Total human genomic DNA was fractionated by agarose gel electrophoresis according to the methods of Southern as described by Maniatis et al. (1982). The digested samples (see above) were loaded into a 0.8% agarose gel. The gel was prepared as follows: Agarose (SeaKem, FMC) was added to 1X TAE (0.04M Tris-acetate, Fisher Scientific; 0.001M EDTA) and microwaved until boiling (~7 minutes). Ethidium bromide (0.5μg/ml Sigma) was added and the solution was cooled to 60°C in a water bath. The cooled agarose was poured into a gel mold (15.5cm X 25.5cm) and a comb was inserted so that there was a minimum of 0.5mm of agarose to seal the bottom of the

Table 12. Probe/restriction enzyme combinations

HGM #	Probe	Chromosomal Location	Restriction Enzyme	PIC	Buffer[a] [salt]	Reaction Temp (°C)
D15S24	CMW-1	15pter-q13	EcoRI	.75	high	37
D15S1	pMS1-14	15q14-q21	MspI	.37	low	23
D15S2	pDP151	15q15-q22	EcoRI	.33	high	37
D15S27	pTHH55	15q	MspI	.34	low	23
D21S13	pGSM21	21pter-q21.1	TaqI	.33	med	65
			PstI	.27	med	37
D21S16	pGSE9	21q11.2-q21	NciI	.16	high	37
			XbaI	.07	high	37
D21S26	26C	21pter-q21.1	PstI	.35	med	37
			BglII	.56	high	37
D21S24	p21.3	21q21-qter	PstI	.34	med	37
D21S112	CRI-L427	21	RsaI	.93	low	37
D21S15	pGSE8	21q22.3	MspI	.37	low	23
D21S19	pGSB3	21q22.3-qter	PstI	.07(A) .27(B)	med	37

[a] Buffer Components

Buffer	NaCl	Tris-Cl	$MgCl_2$
low	0	10mM	10mM
med	50mM	10mM	10mM
high	100mM	50mM	10mM

wells. After the gel set (~45 minutes), the combs were removed and the gel was placed into the electrophoresis tank (DNA Subcell, BioRad). About 1l of 1X TAE buffer was added until the gel was covered. After the samples were loaded into the wells, electrophoresis was conducted for 18 hours at 40-50 volts. At completion, the gel was photographed with ultra-violet light (254nm) and exposed further for 45 seconds. The gel was rocked gently for 1 hour at room temperature in 1l of denaturation solution (0.5N NaOH, 1.5N NaCl) and rinsed twice with deionized water. The gel was then rocked for 1 hour at room temperature in 1l of neutralization solution (0.5M Tris-HCl, pH 8.0, 1.5N NaCl).

The transfer of the DNA from the gel to a nylon filter was accomplished by the methods of Southern (1975) with the following modifications. The components of the transfer apparatus were soaked in 10X SSC (20X SSC: 3M NaCl, 0.3M sodium acetate, pH 7.0) and stacked on top of one another in the following order: 5 plastic pipets were placed in the bottom of a glass baking dish; 3 sponges; 4 sheets of filter paper (3MM Chr Whatman); the agarose gel; transparency with cut out exposing samples; nylon filter (Nytran); 2 sheets of filter paper (3MM Chr Whatman); 2 stacks of paper towels; plexiglass sheet and 500g weight. About 500ml of 10X SSC was added to the dish and the transfer proceeded for ~12 hours. The transfer apparatus was disassembled and the filter was washed in 2X SSC for 15 minutes (2 times) then baked at 80°C in a vacuum oven (Napco) for 2 hours.

X. Probe preparation and filter hybridization

Competent cells (BRL) were transformed according to the modifications of methods of Hanahan (1983). Twenty microliters of E. coli cells were placed into a sterile Eppendorf tube and 1μl of undiluted plasmid DNA was added and put on ice for 30 minutes. The cells were heat shocked to take up the DNA by placing them in a 42°C water bath for 40 seconds and placed on ice. Eighty microliters of SOC (2% bactotryptone, DIFCO; 0.5% yeast extract, DIFCO; 10mM $MgSO_4$, Fisher Scientific; 10mM $MgCl_2$, Fisher Scientific; 20mM glucose, Sigma) was added and the mixture was shaken at 37°C for 1 hour. The cells were plated on bacto-agar plates (LB media: 10g bactotryptone, 5g bacto-yeast extract, 10g NaCl with 5g bacto-agar) with 50μg/ml ampicillin or 15μg/ml tetracycline. Only those bacteria which took up the plasmid containing the vector, insert and selectable resistance marker grew on the plates (see Table 13 for insert/vectors). The following day, one colony for each probe was selected. The colony was grown in 5ml LB media overnight. The next day, 4.5ml of a saturated culture was added to 250ml LB media and grown overnight at 37°C in a shaking incubator. After the incubation period, 0.5ml of culture was frozen in 50% glycerol for storage.

The plasmid purification procedure used was a modification of the methods of Birnborm and Doly (1979). At the completion of the overnight culture (~16 hours), the flasks were placed on ice for 30 minutes. Cells were pelleted

at 5000 rpm at 4°C for 15 minutes. The cells were resuspended in 6ml freshly prepared lysis solution (25mM Tris-HCl, pH 7.5; 10mM EDTA; 15% sucrose; 4mg/ml lysozyme, Sigma) and incubated on ice for 20 minutes. Twelve milliliters of a 0.2M NaOH and 1% SDS solution was added, mixed by inversion and incubated on ice for 10 minutes. Then 7.5ml of 3M sodium acetate pH 4.6 was added and mixed by inversion and incubated on ice for 20 minutes. The mixture was centrifuged at 1500 rpm for 15 minutes. The supernatant was decanted and 50μl of RNase (10mg/ml stock) was added and incubated for 20 minutes at 37°C. The pellet (large chromosomal DNA and cellular debris) was discarded. After the incubation, an equal volume of a 1:1 phenol:chloroform solution was added, the mixture was rocked for 10 minutes at room temperature and centrifuged for 10 minutes at 1500 rpm. The extraction procedure was repeated once. Two volumes of 100% ethanol was added to the precipitate and placed in a -70°C freezer overnight. The plasmid DNA was pelleted by centrifugation at 10,000 rpm for 30 minutes. The crude DNA pellet was washed with 70% ethanol and the pellet was dried in a vacuum for 15 minutes. The pellet was resuspended in 1.8ml of a 10mM Tris-Hcl, pH 8.0, 1mM EDTA, pH 8.0 and 1mM NaCl and the pellet was allowed to go into solution in a 60°C water bath for 30 minutes. The sample was loaded onto the top of a pZ523 column (5prime-3prime Inc.) and centrifuged at 2500 rpm for 13 minutes. The purified plasmid was added to 0.6X volume of 100% isopropanol and incubated at room temperature for 20 minutes. The mixture

was centrifuged at 10,000 rpm for 30 minutes. The supernatant was discarded and the pellet washed twice with 1ml of 70% ethanol. The pellet was dried in a vacuum and then resuspended in 1ml TE, pH 7.5 and placed in the 60°C water bath for 30 minutes. The concentration of the plasmid was determined in μg/ml using a Shimadzu spectrophotometer at UV 260nm. The plasmid was digested with the appropriate enzymes to cut the insert from the vector using the appropriate temperature and buffer conditions (Table 13). Ten percent loading buffer was added and the samples were loaded into a 1% low melting temperature agarose gel(SeaPlaque, FMC) with 0.5μg/ml ethidium bromide and fractionated at 90 volts for 1 hour or until good separation between the vector and insert fragments was achieved. The insert fragment was cut from the gel, weighed, 3 times the weight in sterile water was added, and the insert/gel/water mixture was boiled for 7 minutes and cooled at 37°C for 10 minutes. Each probe was labelled with ^{32}PdCTP by the Hexamer-labelling procedure (Feinberg and Vogelstein, 1983; 1984) prior to use. The DNA insert (31μl) was incubated at room temperature for at least 2 hours with 2μl bovine serum albumin (BRL), 2μl Klenow fragment (Pharmacia), 5μl ^{32}PdCTP (Dupont,~50μCi) and 10μl of OLB buffer (1.25M Tris-HCl, 0.125M $MgCl_2$, 18μl 2-mercaptoethanol, 5μl 0.1M dATP, 5μl 0.1M dGTP, 5μl 0.1M dTTP, 2M Hepes, 90 OD u/ml hexamer polynucleotides in TE). The reaction was stopped with 200μl of stopping solution (20mM NaCl, 20mM Tris-HCl, 2mM EDTA, 0.25% SDS, 1μM CTP). The incorporation of the ^{32}P was

Table 13. Conditions for selecting DNA inserts

Probe	Insert Size(Kb)	Vector[a]	Selectable Marker[b]	Enzyme	Buffer[c]
D15S1	2.9	pBR322	amp	EcoRI/HindIII	high/med
D15S2	2.6	pBR322	amp	EcoRI/HindIII	high/med
D15S24	3.8	pUC18	amp	EcoRI	high
D15S27	7.0	pUC18	amp	BamHI	med
D21S13	9.0	pUC9	amp	EcoRI	high
D21S15	6.3	pUC9	amp	EcoRI	high
D21S16	7.0	pUC9	amp	EcoRI	high
D21S19	6.4	pUC9	amp	EcoRI	high
D21S24	3.5	pAT153	tet	PstI	med
D21S26	2.5	pAT153	tet	PstI	med
D21S112	10-20	Lambda Charon 4A (phage preparation was used)			

[a]vector sizes: pBR322=4.4Kb; pUC18=2.7Kb; pUC9=2.7Kb; pAT153=3.6Kb

[b]amp=ampicillin, tet=tetracycline

[c]see Table 12 for buffer descriptions

checked by making a 1:10 dilution of the probe in water. a 5μl aliquot was placed directly on filter paper (24mm GF/C Whatman) and placed under a heat lamp to dry. Five microliters were placed in 5ml cold 10% trichloroacetic acid (TCA) (Fisher Scientific) with 100μl of 20mM EDTA/0.5mg/ml salmon sperm DNA (Sigma). After a 15 minute incubation on ice, the mixture was filtered through filter paper (24mm GF/C Whatman), rinsed with 95% ethanol and dried under a heat lamp. The two filters were each placed in heat-sealed filmware (Nalgene) with 3ml of counting solution (Toluene, Beckman) and counted in a scintillation counter (LKB). The ratio of the TCA-precipitated cpms to the total cpms determined the percent incorporation. The TCA-precipitated counts (incorporated) were used to calculate $2X10^7$ counts per minute, which was added to an equal volume of 1.0N NaOH for 5 minutes in order to denature the double stranded probe. The entire incubated sample was then added to the prepared filter.

The baked filter was pre-washed in 500ml 0.1X SSC and 1% SDS in a 67°C shaking water bath for 1 hour. The filter was prehybridized in 60ml of a solution of 6X SSC, 1X Denharts (5g Ficoll, Sigma; 5g polyvinylpyrrolidone, Sigma; 5g BSA fraction V, Sigma) and 0.25mg/ml sonicated salmon sperm DNA solution at 60°C in a plastic pouch (Dazey) for 4 hours to overnight. The pouch was cut in one corner and the prehybridization solution was discarded. The filter was hybridized in 10ml of fresh prehybridization solution, 10% dextran sulfate, and $2X10^7$ cpm/filter of NaOH denatured probe, sealed with a hot

iron and incubated at 60°C overnight. After the incubation, the pouch was cut with scissors and the solution was discarded into a radioactive liquid waste container. The filter was placed into a series of 3 washes: (wash 1) 5 minutes at room temperature in 2X SSC and 0.5% SDS; (wash 2) 15 minutes at room temperature in 2X SSC and 0.1% SDS with shaking; (wash 3) 2 hours at 60°C in 0.1X SSC and 0.5% SDS with shaking. The filters were blotted dry with filter paper (3MM Chr Whatman) and wrapped in plastic wrap (Reynolds). The filters were placed on Kodak XAR film for overnight exposure or Kodak XRP film for a 2-3 day exposure in a light tight cassette (Fotodyne) with Intensifying screen (Fotodyne) in a -70°C freezer.

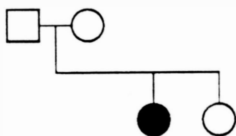
The filters could be reused by washing in 0.2N NaOH for 20 minutes at 67°C followed by washing in 0.1X SSC, 1.0% SDS, 0.2M Tris-Hcl, pH 7.5 for 30 minutes at 67°C. The filters were placed in the prehybridization solution and treated as above.

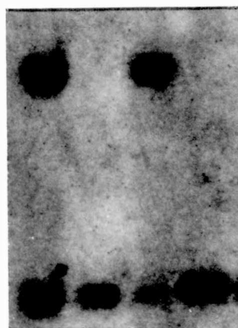
XI. Interpretation of the Autoradiographs for parental origin assignment, recombination and isochromosome identification

Parental origins of the de novo rearrangements were assigned according to informative RFLPs. The copy number of each probe was determined in the proband by comparison of band intensities to normal heterozygotes and homozygotes (their noncarrier parents and siblings) (Fig. 8). After each probe/restriction enzyme combination was performed for a family, the chromosomal haplotypes were constructed.

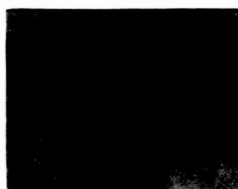
Fig. 8. The use of restriction fragment length polymorphisms for the parental origin assignment of _de novo_ acrocentric rearrangements. The autoradiograph results of Southern hybridizations for families 1 and 20 are shown. For each family, the upper autoradiographs demonstrate informative markers (family 1, paternal; family 20, maternal). The lower autoradiographs show patterns indicative of isochromosomes as the probands are homozygous for these markers and the parents are heterozygous.

Family 1



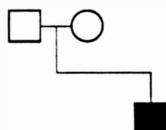


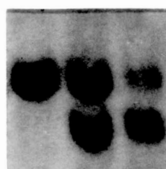
D21S19/Pst I



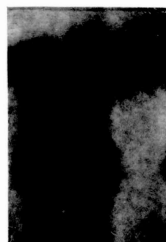
D21S112/Rsa I

Family 20





D21S13/Taq I



D21S26/Pst I

The molecular markers were arranged relative to their mapped positions (Petersen et al., 1989; Meijer et al., 1989). It was assumed that D21S13 was the closest marker to the centromere 21. The chromosome 21 map assignment used was cen->D21S13->D21S16->D21S26->D21S24->D21S112->D21S15->D21S19 ->qter. Markers D21S15 and D21S19 were used to assess recombination between the proximal markers D21S13 and D21S26 and/or the cytogenetic markers.

In the probands with homologous rearrangements, the rearrangements were assumed to be Robertsonian translocations between one parental set of homologous chromosomes 21. If the parent of origin was heterozygous for the "centromeric" marker D21S13 and the proband was heterozygous for D21S13, then the proband was assumed to have a Robertsonian translocation. However, if the parent of origin was heterozygous for D21S13 and the proband was homozygous for D21S13, then the proband was assumed to have an isochromosome 21.

XII. Statistical Analyses

The distributions of all variables were examined using the univariant analysis program from the Statistical Analysis System (SAS) computer programs to test if the data approximated normal distributions. All significance levels were set at α=0.05.

The sex distribution of the parental origins of <u>de novo</u> rearrangements and the incidence of dNOR variants among the study groups were tested using the chi-square goodness of fit

test. The mean total NOR scores, mean number of silver positive chromosomes, mean number of satellite associations, mean number of chromosomes in satellite associations, and mean number of chromosomes per satellite association were compared between the study groups using a Duncan's new multiple-range test and analysis of variance (ANOVA). The frequency distributions of the NOR scores for all acrocentric chromosomes were obtained for each study group and compared to a random distribution using the chi-squared goodness of fit test. The frequency of the pairwise satellite associations was obtained for each study group and compared to random using a chi-square goodness of fit test. The random expectations were obtained by considering all possible chromosome pairings. Homolog-homolog associations could occur only one way (1/45) (i.e.13A/13B) while nonhomolog-nonhomolog associations could occur in 4 ways (4/45) (i.e. 13A/14A, 13A/14B, 13B/14A and 13B/14B). These probabilities were multiplied by the total number of pairwise satellite associations observed in order to calculate the expected pairwise associations for each.

The satellite association indices (AI) were calculated for each acrocentric chromosome as the total number of the specific chromosome in satellite associations as divided by the total number of the specific chromosome per cell (Hansson and Mikkelsen, 1978). This corrected for individuals who carry translocations and thus had fewer chromosomes able to participate in satellite associations. The AIs were compared

between the study groups by an ANOVA and Duncan's new multiple-range test.

The final analyses were within the study group of the parents of origin. The parents were divided between dNOR (+) and dNOR (-) status and between contributors of true Robertsonian translocations and homologous rearrangements. Comparisons in mean NOR score, mean number of silver positive chromosomes, mean number of satellite associations, mean number of chromosomes in satellite associations and mean number of chromosomes per satellite association were made using ANOVA and Duncan's new multiple-range test.

RESULTS

I. The acrocentric rearrangements: Description of the study populations

Twenty-six probands who carry de novo acrocentric rearrangements were ascertained for this study (Table 14). Twenty-five probands had a Robertsonian-type rearrangement and one proband had a "mirror image" rearrangement t(21;21)(q22;q22) (proband 26). Four probands were "balanced" translocation carriers and all four had Robertsonian translocations between nonhomologous chromosomes (probands 2, 9, 11 and 25). An additional proband, 7, who had Down syndrome, had a "balanced" de novo rob(13q14q) plus an extra chromosome 21. Of the 20 probands who were "unbalanced", 18 had Down syndrome, one had Patau syndrome (proband 4) and one was trisomic for chromosome 15 (proband 8). Of the probands with Down syndrome, 11 had apparent rearrangements between homologous chromosomes 21 [rea(21q21q)]. It was not known prior to this study if these probands had rob(21q21q) or i(21q). Seven probands who had Down syndrome had rearrangements between nonhomologous chromosomes: one rob(13q21q) (proband 23), five rob(14q21q) (probands 3, 10, 12, 15 and 24) and one rob(15q21q) (proband 16).

Table 14. Rearrangements found in 26 probands ascertained for de novo Robertsonian translocations

Proband/Family #	Karyotype	Diagnosis
1	46,XX,dic(21;21)(p11;p11)	Down syndrome
2	45,XY,rob(13q14q)	balanced translocation carrier
3	46,XX,rob(14q21q)	Down syndrome
4	46,XY,rob(13q14q)	Patau syndrome
5	46,XY,rea(21q21q)*	Down syndrome
6	46,XY,rea(21q21q)	Down syndrome
7	46,XX,rob(13q14q),+21	Down syndrome
8	46,XY,dic(15;15)(p11;p11)	Trisomic for chromosome 15
9	45,XY,rob(13q14q)	balanced translocation carrier
10	46,XX,rob(14q21q)	Down syndrome
11	45,XY,rob(13q15q)	balanced translocation carrier
12	46,XX,rob(14q21q)	Down syndrome
13	46,XY,rea(21q21q)	Down syndrome
14	46,XX,rea(21q21q)	Down syndrome
15	46,XY,rob(14q21q)	Down syndrome
16	46,XY,rob(15q21q)	Down syndrome
17	46,XY,rea(21q21q)	Down syndrome
18	46,XX,rea(21q21q)	Down syndrome
19	46,XY,dic(21;21)(p11;p11)	Down syndrome
20	46,XY,rea(21q21q)	Down syndrome
21	46,XX,dic(21;21)(p11;p11)	Down syndrome
22	46,XX,rea(21q21q)	Down syndrome
23	46,XX,rob(13q21q)	Down syndrome
24	46,XX,rob(14q21q)	Down syndrome
25	45,XY,rob(15q21q)	balanced translocation carrier
26	46,XX,dic(21;21)(q22;q22)	Down syndrome

* rea denotes those rearrangements in which it was not known whether the rearrangement was an isochromosome or a "true" Robertsonian translocation.

The distribution of all Robertsonian-type rearrangements was examined (Table 15). The distribution differed significantly from random (X^2_{14}=214.03, p<0.0001). This difference could be due to the over-representation of rea(21q21q) in the Down syndrome population. When only the nonhomologous rearrangements were examined and all rearrangements were assumed equally probable, the distribution differed significantly from random as a results of the over-representation of rob(13q14q) and rob(14q21q) and the under-representation of rob(Dq22q) and rob(21q22q) (X^2_9=23.15, 0.01>p>0.005) (Table 16).

Twenty-five individuals were ascertained as balanced carriers of familial Robertsonian translocations. All individuals were found to carry translocations between nonhomologous chromosomes (Table 17). The distribution of the rearrangements differed significantly from random (X^2_{14}=60.14, p<0.0001). There was an over-representation of rob(13q14q) and rob(14q21q) in this population.

When the familial and de novo populations were combined, the distribution differed significantly from random (X^2_{14}= 152.65, p<0.0001) (Table 18). The rearrangements rob(13q14q), rob(14q21q) and rob(21q21q) were over-represented among the possible rearrangements. When the percent frequency distributions from this study (Table 19) were compared to the distribution of 1471 Robertsonian translocations compiled by Therman et al. (1989) (Table 2), there was no significant difference between the two distributions (X^2_{14}=23.26,

0.10>p>0.05).

As shown in Table 14, C-banding revealed two centromeres in the rearrangements of five probands (1, 8, 19, 21 and 26). DAPI-Distamycin staining revealed two short arms in the rearrangement in proband 8 [dic(15;15)(p11;p11)].

II. Parental origin assignments of the _de novo_ rearrangements

A. Cytogenetic heteromorphisms

By using QFQ, NOR and morphological heteromorphisms, the parental origins were determined in 21/25 (84%) of the _de novo_ Robertsonian-type rearrangements (Table 20). The parental origin was also determined in the "mirror image" chromosome 21 rearrangement. The parental origins could not be determined using cytogenetic heteromorphisms in four families. In family 10, all family members had low mitotic indexes. In three families (14, 18, and 22), the probands inherited a free-lying chromosome 21 that was indistinguishable from either the maternal or paternal complements. Therefore, the parental origins were determined for 12/13 (92.3%) _de novo_ Robertsonian translocations and 9/12 (75%) _de novo_ homologous rearrangements.

When the _de novo_ nonhomologous ("true") Robertsonian translocations (n=13) were combined with the homologous Robertsonian-type rearrangements (n=12), there was no significant difference between maternally derived (n=11) and paternally derived (n=10) _de novo_ rearrangements (X^2_1=0.048, p>.90) (Table 21). Likewise, there were no significant differences in parental origins within "true" Robertsonian

Table 15. Distribution of de novo Robertsonian translocations in this study [observed(expected)]

	Chromosomes				
	13	14	15	21	22
13	0(0.56)	-	-	-	-
14	4(2.22)	0(0.56)	-	-	-
15	1(2.22)	0(2.22)	1(0.56)	-	-
21	1(2.22)	5(2.22)	2(2.22)	11(0.56)	-
22	0(2.22)	0(2.22)	0(2.22)	0(2.22)	0(0.56)

$X^2_{14}=214.03$, $p<0.0001$, distribution is nonrandom

Table 16. Distribution of de novo nonhomolgous Robertsonian translocations in this study [observed(expected)]

	Chromosomes				
	13	14	15	21	22
13	-	-	-	-	-
14	4(1.3)	-	-	-	-
15	1(1.3)	0(1.3)	-	-	-
21	1(1.3)	5(1.3)	2(1.3)	-	-
22	0(1.3)	0(1.3)	0(1.3))	0(1.3)	-

$X^2_9=23.15$, $0.01>p>0.005$, distribution is nonrandom

Table 17. Distribution of familial Robertsonian translocations in this study [observed(expected)]

	Chromosomes				
	13	14	15	21	22
13	0(0.56)	-	-	-	-
14	10(2.22)	0(0.56)	-	-	-
15	0(2.22)	0(2.22)	0(0.56)	-	-
21	1(2.22)	9(2.22)	1(2.22)	0(0.56)	-
22	1(2.22)	2(2.22)	0(2.22)	1(2.22)	0(0.56)

$X^2_{14} = 60.14$, $p < 0.0001$, distribution is nonrandom

Table 18. Distribution of combined familial and de novo Robertsonian-type rearrangements in this study [observed(expected)]

	Chromosomes				
	13	14	15	21	22
13	0(1.11)	-	-	-	-
14	14(4.44)	0(1.11)	-	-	-
15	1(4.44)	0(4.44)	1(1.11)	-	-
21	2(4.44)	14(4.44)	3(4.44)	11(1.11)	-
22	1(4.44)	2(4.44)	0(4.44)	1(4.44)	0(1.11)

$X^2_{14} = 152.65$, $p < 0.0001$, distribution is nonrandom

Table 19. Frequencies of combined familial and de novo Robertsonian-type rearrangements in this study (percent)

	Chromosomes				
	13	14	15	21	22
13	0(0)	-	-	-	-
14	14(28)	0(0)	-	-	-
15	1(2)	0(0)	1(2)	-	-
21	2(4)	14(28)	3(6)	11(22)	-
22	1(2)	2(4)	0(0)	1(2)	0(0)

In a comparison with Therman et al., 1989 (Table 2), there is no significant difference between the two distributions, $X^2_{14}=23.26$, $0.10>p>0.05$.

Table 20. Assignment of parental origins based on cytogenetic heteromorphisms

Family No.	Chrom	Mother	Father	Proband	Informative Heteromorphisms*	Parental Origin of Rearrangement
1	21	a b	c d	b	QFQ, NOR	paternal
2	13	a b	c d	c	QFQ	maternal
	14	a b	c c	c	NOR	maternal
3	14	a b	c d	a	QFQ, M	paternal
	21	a b	c d	a c	NOR	indeterminate
4	13	a b	c d	b d	NOR	indeterminate
	14	a b	c c	c	M	maternal
5	21	a b	c d	a	QFQ, NOR	paternal
6	21	a b	c d	a	QFQ, NOR	paternal
7	13	a b	c d	d	QFQ	maternal
	14	a b	c d	d	QFQ	maternal
	21	a b	a c	a a d	none	indeterminate
8	15	a a	c d	a	M	paternal
9	13	a b	c d	a	QFQ, NOR	paternal
	14	a b	c c	b	QFQ, NOR	paternal
10	14				none	low mitotic index
	21				none	low mitotic index
11	13	a b	c d	d	NOR	maternal
	15	a a	c d	c	NOR	maternal
12	14	a b	c d	c	NOR	maternal
	21	a a	a a	a a	none	indeterminate
13	21	a b	c d	d	QFQ	maternal
14	21	a b	a b	a	none	uninformative
15	14	a b	c d	d	QFQ, NOR	maternal
	21	a b	c d	b c	QFQ, NOR	indeterminate
16	15	a b	c d	b	QFQ, NOR, M	paternal
	21	a b	c c	b c	QFQ, NOR	indeterminate
17	21	a b	c d	c	QFQ	maternal
18	21	a a	a b	a	none	indeterminate
19	21	a b	c d	c	QFQ, M	maternal

Table 20. continued.

20	21	a b	c d	c	QFQ, NOR	maternal
21	21	a b	c c	a	QFQ, NOR, M	paternal
22	21	a b	a c	a	none	indeterminate
23	13	a b	b c	a	QFQ, M	paternal
	21	a b	b c	b b	none	indeterminate
24	14	a a	c c	c	NOR	maternal
	21	a a	a b	a a	none	indeterminate
25	15	a b	c c	b	QFQ, NOR	paternal
	21	a b	c d	a	QFQ, NOR	paternal
26	21	a b	c d	b c c	NOR, M	paternal

* QFQ=Q-bands by fluorescence with quinacrine
NOR=Nucleolar Organizer Region with ammoniacal silver stain
M=Morphology

Table 21. Summary of assignments of parental origin based on cytogenetic heteromorphisms

Rearrangement	Maternal	Paternal	Indeterminate
rob(13q14q)	3	1	0
rob(13q15q)	1	0	0
rob(13q21q)	0	1	0
rob(14q21q)	3	1	1
rea(15q15q)	0	1	0
rob(15q21q)	0	2	0
rea(21q21q)	4	4	3
Total	11	10	4

Combined: $X^2_1=0.048$, $0.95>p>0.90$

"true" Robertsonian translocations: $X^2_1=0.33$, $0.75>p>0.50$

Homologous rearrangements: $X^2_1=0.11$, $0.75>p>0.50$

translocations (maternal=7, paternal=5, X^2_1=0.33) or within homologous rearrangements (maternal=4, paternal=5, X^2_1=0.11) (Table 21). Additionally, the parental origin of the "mirror image" chromosome 21 rearrangement in proband 26 was determined to be paternally derived.

The utility of the cytogenetic heteromorphisms in parental origin assignments was examined (Table 22). Of the 22 informative families, 36% (8/22) of the parental origins were determined using only one heteromorphism (QFQ, NOR or morphological) and 64% (14/22) were determined based on two or more heteromorphisms. Overall, the use of two or more heteromorphisms nearly doubled (1.75X) the ability to determine the parental origins of _de novo_ acrocentric rearrangements. Additionally, the utility of the cytogenetic heteromorphisms was examined for a total of 49 free-lying chromosomes from the probands (Table 22). The chromosomes shown in Table 20 were the free-lying homologs of the chromosomes involved in the rearrangements. For example, in family 1, the proband had a rea(21q21q) and the QFQ and NOR heteromorphisms for the free-lying chromosome 21 were informative for assigning the parental origin. In another case, family 2, the proband had a rob(13q14q) and free-lying chromosomes 13 and 14 for which the informative heteromorphisms, QFQ and NOR respectively, were used to determine the parental origin. These heteromorphisms have been tabulated in Table 22. Of the 37 informative chromosomes, 43% (16/37) of the parental origins were

Table 22. Utility of cytogenetic heteromorphisms in parental origin determination of *de novo* acrocentric rearrangements

Heteromorphism*	Families	free-lying chromosomes
QFQ only	3	5
NOR only	3	9
M only	2	2
QFQ and NOR	8	13
QFQ and M	3	3
NOR and M	1	3
QFQ, NOR and M	2	2
none	4	12
Total	26	49

* QFQ = Q-bands by fluorescence with quinacrine
NOR = Nucleolar Organizer Region with ammoniacal silver stain
M = Morphology

determined using only one heteromorphism (QFQ, NOR or morphological) and 57% (21/37) were determined based on two or more heteromorphisms. Overall, the use of two or more heteromorphisms increased the ability to assign the parental origin of an individual chromosome by 1.3X. Twenty-four percent (12/49) of the chromosomes could not be assigned based on the cytogenetic heteromorphisms alone, representing one or more of the free-lying chromosomes in seven families. All uninformative chromosomes were chromosomes 21 (12/12). However, except for the three families mentioned previously in whom the parental origins could not be assigned (excluding family 10 with low mitotic indexes), the origins were assigned for the de novo nonhomologous rearrangements using the non-translocated free-lying homologous chromosomes in the remaining four families.

The cytogenetic heteromorphisms of all free-lying acrocentric chromosomes were evaluated to rule-out nonpaternity. In family 7, the proband inherited a chromosome 21 that was not present in either parent (Table 20). Chromosome "d" was apparently 21p- using QM and ammoniacal silver stains. Chromosome "d" was determined to maternal in origin based on RFLP analyses (Fig. 10). The p-arm, NOR and satellites of the chromosome 21 "d" may have been lost during the formation of the de novo rob(13q14q). The cytogenetic heteromorphisms for the free-lying chromosomes 13 and 14 were consistent with the paternal chromosomes. Therefore, the rearrangement was maternally derived. There were no other

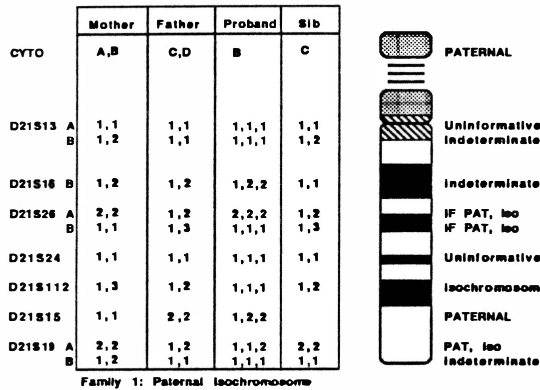
inconsistencies observed between the parental and proband complements in the other 25 families.

B. Restriction fragment length polymorphisms (RFLPs)

DNA was obtained for 14 families and the results of the RFLP analyses are shown in Figures 9 and 10. The parental origin assignments based on the cytogenetic and RFLP analyses were compared in Table 23. The parental origins were assigned in the four families in which the cytogenetic heteromorphisms were indeterminate (families 10, 14, 18 and 22). In two families in which both probands have "true" Robertsonian translocations, the parental origins could not be assigned based on the loci tested (families 12 and 16). Construction of the haplotypes for chromosome 21 in the parents, probands and two normal siblings (family 16) was not helpful in the assignment of these parental origins. In the remaining eight families, the cytogenetic and RFLP parental origin assignments agreed in every case. Therefore, the origins of the de novo rearrangements (and extra chromosome 21 in family 7), were assigned by RFLP analysis in 12/14 (85.7%) cases studied: 8 homologous rearrangements, 3 Robertsonian translocations and 1 trisomy 21. Additionally, the rea(21;21)(q22;q22) in the proband of family 26 was confirmed to be paternally derived.

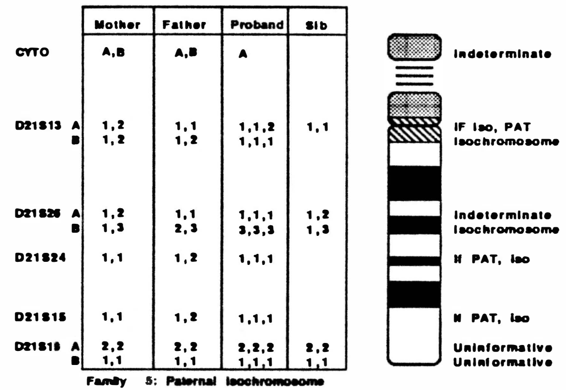
When the cytogenetic and RFLP results were combined, the parental origins were assigned in all families (n=26) (Table 24). There was no significant difference in the sex distribution of the origins in 1) the combined rearrangements (X^2_1=0.04); 2) "true" Robertsonian translocations (X^2_1=0.69);

Fig. 9. Haplotypes for 8 families in which the proband has Down syndrome and a _de novo_ rearrangement of chromosome 21. (Adapted from Stewart et al., 1988.) Cytogenetic heteromorphisms, RFLPs and assignments are shown. All probands were determined to have isochromosomes. In family 22, crossing over occurred between markers D21S13 and D21S26 in the father as indicated.



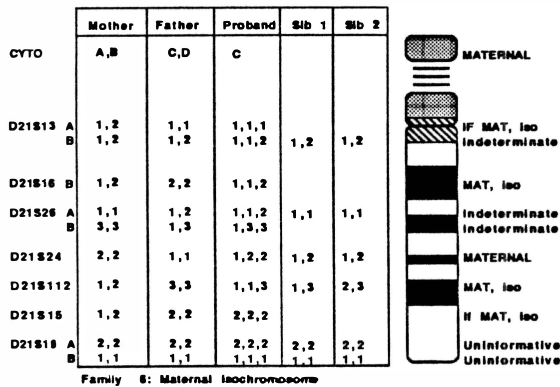
	Mother	Father	Proband	Sib	
CYTO	A,B	C,D	B	C	PATERNAL
D21S13 A	1,1	1,1	1,1,1	1,1	Uninformative
B	1,2	1,1	1,1,1	1,2	Indeterminate
D21S16 B	1,2	1,2	1,2,2	1,1	Indeterminate
D21S26 A	2,2	1,2	2,2,2	1,2	IF PAT, iso
B	1,1	1,3	1,1,1	1,3	IF PAT, iso
D21S24	1,1	1,1	1,1,1	1,1	Uninformative
D21S112	1,3	1,2	1,1,1	1,2	Isochromosome
D21S15	1,1	2,2	1,2,2		PATERNAL
D21S19 A	2,2	1,2	1,1,2	2,2	PAT, iso
B	1,2	1,1	1,1,1	1,1	Indeterminate

Family 1: Paternal isochromosome



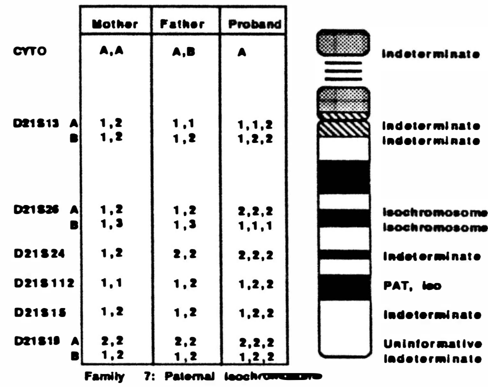
	Mother	Father	Proband	Sib	
CYTO	A,B	A,B	A		Indeterminate
D21S13 A	1,2	1,1	1,1,2	1,1	IF iso, PAT
B	1,2	1,2	1,1,1		Isochromosome
D21S26 A	1,2	1,1	1,1,1	1,2	Indeterminate
B	1,3	2,3	3,3,3	1,3	Isochromosome
D21S24	1,1	1,2	1,1,1		H PAT, iso
D21S15	1,1	1,2	1,1,1		H PAT, iso
D21S19 A	2,2	2,2	2,2,2	2,2	Uninformative
B	1,1	1,1	1,1,1	1,1	Uninformative

Family 5: Paternal isochromosome



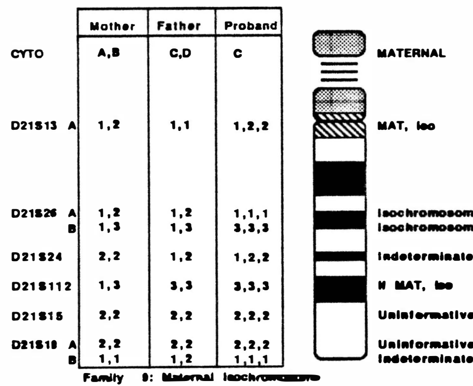
	Mother	Father	Proband	Sib 1	Sib 2	
CYTO	A,B	C,D	C			MATERNAL
D21S13 A	1,2	1,1	1,1,1			IF MAT, iso
B	1,2	1,2	1,1,2	1,2	1,2	Indeterminate
D21S16 B	1,2	2,2	1,1,2			MAT, iso
D21S26 A	1,1	1,2	1,1,2	1,1	1,1	Indeterminate
B	3,3	1,3	1,3,3			Indeterminate
D21S24	2,2	1,1	1,2,2	1,2	1,2	MATERNAL
D21S112	1,2	3,3	1,1,3	1,3	2,3	MAT, iso
D21S15	1,2	2,2	2,2,2			H MAT, iso
D21S19 A	2,2	2,2	2,2,2	2,2	2,2	Uninformative
B	1,1	1,1	1,1,1	1,1	1,1	Uninformative

Family 6: Maternal isochromosome



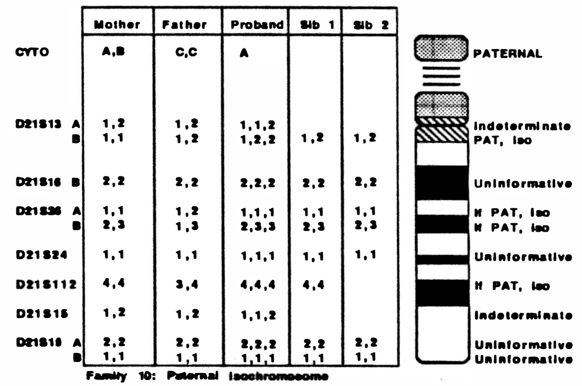
	Mother	Father	Proband	
CYTO	A,A	A,B	A	Indeterminate
D21S13 A	1,2	1,1	1,1,2	Indeterminate
B	1,2	1,2	1,2,2	Indeterminate
D21S26 A	1,2	1,2	2,2,2	Isochromosome
B	1,3	1,3	1,1,1	Isochromosome
D21S24	1,2	2,2	2,2,2	Indeterminate
D21S112	1,1	1,2	1,2,2	PAT, iso
D21S15	1,2	1,2	1,2,2	Indeterminate
D21S19 A	2,2	2,2	2,2,2	Uninformative
B	1,2	1,2	1,2,2	Indeterminate

Family 7: Paternal isochromosome



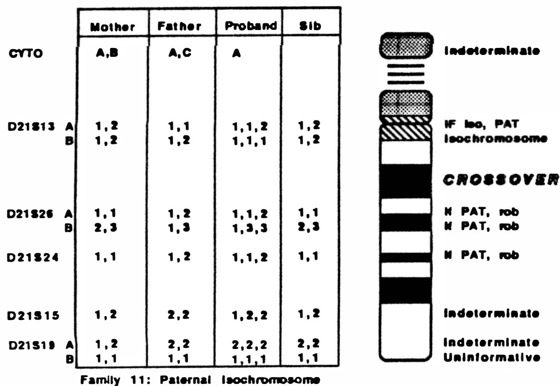
	Mother	Father	Proband	
CYTO	A,B	C,D	C	MATERNAL
D21S13 A	1,2	1,1	1,2,2	MAT, iso
D21S26 A	1,2	1,2	1,1,1	Isochromosome
B	1,3	1,3	3,3,3	Isochromosome
D21S24	2,2	1,2	1,2,2	Indeterminate
D21S112	1,3	3,3	3,3,3	H MAT, iso
D21S15	2,2	2,2	2,2,2	Uninformative
D21S19 A	2,2	2,2	2,2,2	Uninformative
B	1,1	1,2	1,1,1	Indeterminate

Family 9: Maternal isochromosome



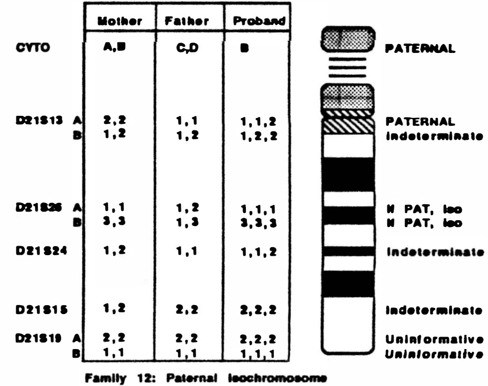
	Mother	Father	Proband	Sib 1	Sib 2	
CYTO	A,B	C,C	A			PATERNAL
D21S13 A	1,2	1,2	1,1,2			Indeterminate
B	1,1	1,2	1,2,2	1,2	1,2	PAT, iso
D21S16 B	2,2	2,2	2,2,2	2,2	2,2	Uninformative
D21S26 A	1,1	1,2	1,1,1	1,1	1,1	H PAT, iso
B	2,3	1,3	2,3,3	2,3	2,3	H PAT, iso
D21S24	1,1	1,1	1,1,1	1,1	1,1	Uninformative
D21S112	4,4	3,4	4,4,4	4,4		H PAT, iso
D21S15	1,2	1,2	1,1,2			Indeterminate
D21S19 A	2,2	2,2	2,2,2	2,2	2,2	Uninformative
B	1,1	1,1	1,1,1	1,1	1,1	Uninformative

Family 10: Paternal isochromosome



	Mother	Father	Proband	Sib	
CYTO	A,B	A,C	A		Indeterminate
D21S13 A	1,2	1,1	1,1,2	1,2	IF iso, PAT
B	1,2	1,2	1,1,1	1,2	Isochromosome
					CROSSOVER
D21S26 A	1,1	1,2	1,1,2	1,1	H PAT, rob
B	2,3	1,3	1,3,3	2,3	H PAT, rob
D21S24	1,1	1,2	1,1,2	1,1	H PAT, rob
D21S15	1,2	2,2	1,2,2	1,2	Indeterminate
D21S19 A	1,2	2,2	2,2,2	2,2	Indeterminate
B	1,1	1,1	1,1,1	1,1	Uninformative

Family 11: Paternal isochromosome



	Mother	Father	Proband	
CYTO	A,B	C,D	B	PATERNAL
D21S13 A	2,2	1,1	1,1,2	PATERNAL
B	1,2	1,2	1,2,2	Indeterminate
D21S26 A	1,1	1,2	1,1,1	H PAT, iso
B	3,3	1,3	3,3,3	H PAT, iso
D21S24	1,2	1,1	1,1,2	Indeterminate
D21S15	1,2	2,2	2,2,2	Indeterminate
D21S19 A	2,2	2,2	2,2,2	Uninformative
B	1,1	1,1	1,1,1	Uninformative

Family 12: Paternal isochromosome

Fig. 10. Haplotypes for 6 families in which the proband has Down syndrome and a *de novo* Robertsonian translocation between nonhomologous chromosomes (Adapted from Stewart et al., 1988). Cytogenetic heteromorphisms, RFLPs and assignments are shown. Crossing over occurred in families 3, 10, 15 and 16 as indicated.

Marker	Mother	Father	Proband	Sib 1	Sib 2	Sib 3	Sib 4	
CYTO 14	A,B	C,D	A					PATERNAL
21	A,B	C,D	A,C	D	D	C	D	Indeterminate
D21S13 A	1,1	1,1	1,1,1	1,1	1,1	1,1	1,1	Uninformative
B	1,2	1,2	1,2,2	2,2	1,2	1,2	1,2	Indeterminate
D21S16 B	1,2	2,2	2,2,2		2,2	1,2	1,2	Indeterminate
								CROSSOVER
D21S26 A	1,2	1,2	1,1,1	1,2	1,2	1,2	1,2	homozygosity
B	1,2	1,2	2,2,3	1,2	1,2	1,2	1,2	? Non-paternity
D21S24	1,2	1,2	1,2,2	2,2	1,2	1,2		Indeterminate
D21S15	1,2	2,2	1,2,2	2,2	1,2	2,2	1,2	If MAT, hetero
D21S19 A	2,2	2,2	2,2,2	2,2	2,2	2,2	2,2	Uninformative
B	1,1	1,1	1,1,1	1,1	1,1	1,1	1,1	Uninformative

Family 3: Paternal rob(14q21q)

Marker	Mother	Father	Proband	
CYTO 13	A,B	C,D	D	MATERNAL
14	A,B	C,D	D	MATERNAL
21	A,B	A,C	A,A,D	Indeterminate
D21S13 A	1,1	1,2	1,1,1	IF PAT, MII
B	1,2	1,2	1,1,2	Indeterminate
D21S26 A	2,2	1,1	1,2,2	MATERNAL
B	1,1	3,3	1,1,3	MATERNAL
D21S24	1,2	1,1	1,1,2	MAT, MII
D21S15	2,2	1,2	1,2,2	IF PAT, MII
D21S19 A	2,2	2,2	2,2,2	Uninformative
B	1,2	1,1	1,1,2	MAT, MII

Family 7: Maternal MII trisomy 21

Marker	Mother	Father	Proband	
D21S13 A	1,2	1,2	1,1,2	Indeterminate
B	1,2	1,1	1,2,2	MAT, hom
D21S16 B	2,2	1,2	1,2,2	IF PAT, hetero
D21S26 A	1,2	1,1	1,1,1	IF MAT, hom
B	1,3	3,3	3,3,3	IF MAT, hom
D21S24	1,2	1,2	2,2,2	IF MAT, hom
				CROSSOVER
D21S112	1,2	3,4	1,2,3	If MAT, hetero
D21S15	1,1	1,2	1,1,2	If PAT, hetero
D21S19 A	2,2	2,2	2,2,2	Uninformative
B	1,1	1,1	1,1,1	Uninformative

Family 10: Maternal rob(14q21q)

Marker	Mother	Father	Proband	
CYTO 14	A,B	C,D	C	MATERNAL
21	A,A	A,A	A,A	Uninformative
D21S13 A	1,2	1,2	1,2,2	Indeterminate
B	1,2	1,2	1,1,2	Indeterminate
D21S16 B	2,2	2,2	2,2,2	Uninformative
D21S26 A	2,2	1,2	2,2,2	Indeterminate
B	1,1	1,3	1,1,1	Indeterminate
D21S112	1,3	2,4	?,?,?	Incomplete
D21S15	2,2	1,2	1,?,?	? Non-maternity
D21S19 A	2,2	2,2	2,2,2	Uninformative
B	1,2	1,1	1,1,1	Indeterminate

Family 12: Maternal rob(14q21q)

Marker	Mother	Father	Proband	Sib 1	Sib 2	
CYTO 14	A,B	C,D	D			MATERNAL
21	A,B	C,D	C,B			Indeterminate
						CROSSOVER
D21S13 A	1,2	2,2	1,1,2	1,2	1,2	MAT, hom
B	1,2	1,2	1,2,?	1,2	1,2	Indeterminate
D21S16 B	1,2	1,2	1,2,?	1,2	1,2	Indeterminate
D21S26 A	2,2	1,2	1,2,2		2,2	If PAT, hetero
B	1,1	1,3	1,1,3	1,3	1,1	If PAT, hetero
D21S24	1,1	1,1	1,1,1	1,1	1,1	Uninformative
D21S112	1,1	2,2	1,1,2	1,2	1,2	MATERNAL
D21S15	1,2	1,1	1,2,2	1,2	1,2	MAT, hom
D21S19 A	2,2	2,2	2,2,2	2,2	2,2	Uninformative
B	1,1	1,1	1,1,1	1,1	1,1	Uninformative

Family 15: Maternal rob(14q21q)

Marker	Mother	Father	Proband	Sib 1	Sib 2	
CYTO 15	A,B	C,D	B			PATERNAL
21	A,B	C,C	B,C			Indeterminate
						CROSSOVER
D21S13 A	1,2	1,2	2,2,2	1,2	1,2	Homozygous
B	1,2	1,2	1,2,?	1,2	1,2	Indeterminate
						CROSSOVER
D21S26 A	1,1	1,2	1,1,2	1,2	1,1	If PAT, hetero
B	3,3	1,3	1,3,3	1,3	3,3	If PAT, hetero
D21S24	1,1	1,1	1,1,1	1,1	1,1	Uninformative
D21S15	1,2	2,2	1,2,2	2,2	2,2	If MAT, hetero
D21S19 A	2,2	2,2	2,2,2	2,2	2,2	Uninformative
B	1,2	1,2	1,2,2	2,2	2,2	Indeterminate

Family 16: Paternal rob(15q21q)

Table 23. Comparison of cytogenetic and RFLP parental origin assignments

Family Number	Parental Origin	
	Cytogenetic	RFLP*
1	paternal	paternal
3	paternal	paternal
7	-	maternal MI
8	paternal	paternal
10	-	maternal
12	maternal	indeterminate
14	-	paternal
15	maternal	maternal
16	paternal	indeterminate
17	maternal	maternal
18	-	paternal
20	maternal	maternal
21	paternal	paternal
22	-	paternal

* All RFLPs were for loci on human chromosome 21 except in family 8 in which RFLPs used were for chromosome 15.

Table 24. Parental origin assignments based on cytogenetic heteromorphisms and RFLP analyses

Rearrangement	Parental Origin	
	Maternal	Paternal
rob(13q14q)	3	1
rob(13q15q)	1	0
rob(13q21q)	0	1
rob(14q21q)	4	1
rea(15q15q)	0	1
rob(15q21q)	0	2
rea(21q21q)	4	7
Total	12	13

Combined: $X^2_1 = 0.04$, $0.9 > p > 0.75$

"true" Robertsonian translocations: $X^2_1 = 0.69$, $0.5 > p > 0.25$

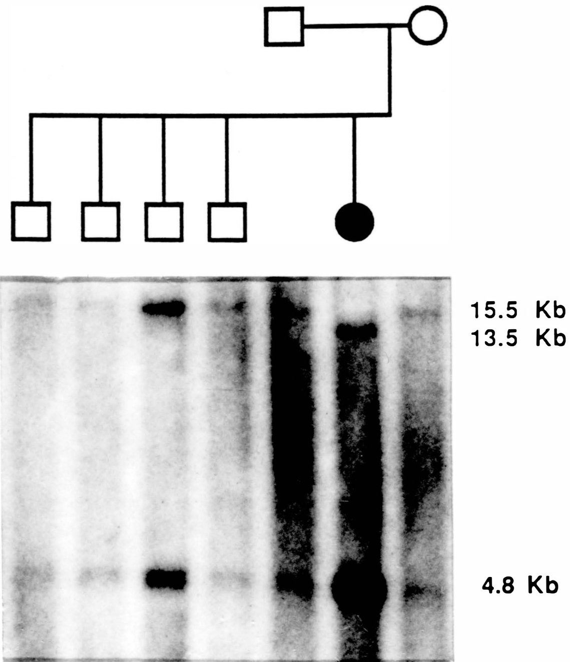
Homologous rearrangements: $X^2_1 = 1.33$, $p = 0.25$

or 3) homologous rearrangements (X^2_1=1.33). Although not statistically significant, there was a tendency for more maternally derived Robertsonian translocations (8/13) and more paternally derived homologous rearrangements (8/12).

In the analyses of the restriction fragment length polymorphisms, two inconsistencies were seen (Fig. 11). First, in family 3, the proband had a fragment not found in either parent. The fragment, ~2Kb smaller, was demonstrated repeatedly with D21S26 and *Bgl*II. No other inconsistencies in inheritance of RFLP or cytogenetic markers were noted in this family and "paternity" testing with non-21 RFLPs were consistent within this family. The altered fragment may have resulted from an altered restriction site at or near the site of the rearrangement. Second, in family 12, D21S15 was repeatedly consistent with non-maternity since the proband is lacking any maternal allele. There were no other inconsistencies with other RFLPs in this family and "paternity" testing with non-21 RFLPs were consistent within this family.

The utility of the RFLPs for parental origin determination of *de novo* acrocentric rearrangements of chromosome 21 was examined (Table 25). The origin could be assigned based on one or more completely informative markers in 64% (9/14) families. An additional 21% (3/14) were assigned based on a combination of markers or construction of haplotypes and in 14% (2/14) families, the parental origins could not be assigned based on the loci tested.

Fig. 11. Pedigrees of families who were found to have inconsistencies in RFLP analyses [families 3 (top) and 12 (bottom)]. In family 3, the proband has a _de novo_ rob(14q21q) and a "new" restriction fragment not observed in either parent. This fragment, ~2Kb smaller, was demonstrated repeatedly with D21S26 (21pter-q21.1) and _Bgl_II. This new fragment may have resulted from an altered restriction site at or near the site of the rearrangement. In family 12, the proband, who has a _de novo_ rob(14q21q), apparently did not inherit an allele from the mother for probe D21S15 (21q22.3) as demonstrated repeatedly with _Msp_I. This finding suggest "non-maternity".


Family 3
15.5 Kb
13.5 Kb
4.8 Kb

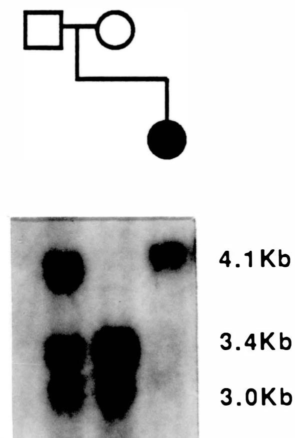

Family 12
4.1Kb
3.4Kb
3.0Kb

Table 25. Utility of RFLPs for determining parental origins

Number of RFLPs parental origin assignment is based	Number of families
One or more informative markers	9
Combined markers or complete haplotype	3
none	2
Total	14

Table 26. Degree of informativeness for each molecular probe

DNA Probe		Number of Families	Informativeness (%)
D21S13	A	3	21.4
	B	2	15.4
D21S16	B	1	14.3
D21S26	A	1	7.1
	B	1	7.1
D21S24		1	7.7
D21S112		4	50.0
D21S15		2	14.3
D21S19	A	1	7.1
	B	0	0.0

Percent informativeness = number of families in which the probe was informative and the parental origin could be assigned, divided by the total number of families tested.

The markers differed in their degree of informativeness. As shown in Table 26, D21S112 was the most informative marker followed by D21S13, D21S16B and D21S15. The remaining markers were equally informative. The degree of informativeness found in this study somewhat mimics each of the probes' PICs (Table 12).

III. Recombination

Recombination could be assessed in 1) the families in which normal siblings were tested or 2) those families in which the rearrangement assignment changed from Robertsonian translocation to isochromosome (or the reverse) due to a detectable recombination (i.e. family 10) [or the nondisjunctional assignment changed from meiosis I to meiosis II in trisomy 21 cases], even in the absence of normal siblings. Recombination was assessed in two ways. First, recombination was examined in the chromosomes inherited by the proband from the parent of origin. Second, recombination was assessed for the normal (non-rearranged) homologs by examining recombination in the chromosomes inherited from both parents.

In families in which the proband had a homologous rearrangement (Fig. 9), recombination was detected in the rearrangement in proband 22 between DNA markers D21S13 and D21S26 in the father and was the only example of detectable recombination in a homologous chromosome 21 rearrangement. Given the results of the RFLP analyses, recombination may have occurred in the father of proband 8 or the rearrangement may

have been an isochromosome (Table 27).

In the families in which the proband had a "true" Robertsonian translocation (Fig. 10), recombination was detected in four probands (3, 10, 15 and 16). It could not be determined if the rearranged or the free-lying chromosome 21 was the recombinant, only that a crossover had taken place in one of the chromosomes contributed by the parent of origin. Recombination could not be detected in the case of trisomy 21 (family 7). Therefore, 55.5% (5/9) of chromosomes tested from the parents of origin had a detectable cross over. The majority of these resulted in "true" Robertsonian translocations in the probands (4/5).

Recombination could be assessed for all chromosomes 21 in eight families who had the normal siblings as well as the probands tested. Recombination was detected in five families (62.5%) (5/8) (families 1, 3, 16, 17, and 22).

IV. Identification of isochromosomes

The molecular and cytogenetic results for the eight families who had a child with a homologous rearrangement 21 are shown in Figure 9. Based on the assumptions given in the Materials and Methods section, part XI, all eight probands were determined to have isochromosomes of chromosome 21: seven i(21q) and one idic(21)(pter->q22). For example in family 1 (Fig. 9.), the de novo rearrangement in the proband was determined to be paternal based on cytogenetic heteromorphisms and the DNA marker D21S15. The rearrangement appeared to be an isochromosome based on the homozygosity in the proband of

Table 27. RFLP analyses of chromosome 15 in family 8

Probe	Mother	Father	Abortus
D15S24	2, 5	2, 3	2, 2, 2
D15S1	1, 2	1, 1	1, 1, 1
D15S2	2, 2	1, 1	1, 1, 2
D15S27	1, 2	1, 1	1, 1, 1

markers D21S26A and B, D21S112 and D21S19A.

From the analyses of the samples obtained from family 8, the 15/15 rearrangement in the abortus was consistent with either an isochromosome 15 or a Robertsonian translocation. The rearrangement was determined to be paternal based on D15S2. However, because of the lack of informativeness of probes D15S27 and D15S1 in this family, the rearrangement could not be distinguished between an i(15q) or a rob(15q15q) with a recombinational event in the father (parent of origin) resulting in homozygosity for D15S24 in the proband (Table 27).

V. Nucleolar Organizer Region

A. Variants

1. Incidence

Double NOR variants (dNORs) were found in 12 parents who had a child with a _de novo_ Robertsonian-type rearrangement; 11 parents of origin and one normal spouse (Table 28). The dNOR variant chromosomes were observed in a significantly higher proportion of families with _de novo_ rearrangements (11/25) than in the control population (5/50) ($p<0.0001$). Two of five dNOR positive (+) control individuals had a history of multiple miscarriages.

When present, the dNOR variant was found to be in the parent in whom the rearrangement originated in all 11 families. Family 6 was uninformative for dNOR involvement since both parents carried a dNOR variant. The rearrangement in the proband was shown by other cytogenetic heteromorphisms

to have been contributed by the father.

There was no significant difference between the distribution of dNOR variants in families with "true" Robertsonian translocations (n=6) and those who had rearrangements involving homologous chromosomes (n=5). Forty-six percent (6/13) of parents who contributed a "true" Robertsonian translocation to their offspring were dNOR(+) and 42% (5/12) parents who contributed a homologous rearrangement were dNOR(+). There was no significant difference in the sex of the carriers of the dNOR variants (male=6, female=6).

The dNOR variants were found on chromosomes 13 (n=1), chromosomes 15 (n=4), chromosomes 21 (n=6) and chromosomes 22 (n=2) in the parents and this distribution was not significantly different from a random distribution for all acrocentric chromosomes (X^2_4=8.92, 0.10>p>0.05). Although not statistically significant, chromosome 21 comprised about 50% of the dNOR(+) chromosomes in the parents.

Among the probands with de novo rearrangements who had dNOR(+) parents (Table 28), four inherited their parents' dNOR(+) chromosome as a free-lying chromosome and four did not inherit the variant chromosome. In one case, demonstrated by RFLP analysis, the dNOR(+) chromosome was involved in the rearrangement, idic(21q) (Fig. 12). In four probands, the inheritance of the dNOR chromosome could not be assessed since it was the same chromosome as the chromosomes in the rearrangement and the heteromorphisms were lost in their formation. RFLP analyses were not performed on these families

because of sample unavailability. Two of 25 carriers of familial translocations were found to carry dNOR variant chromosomes. The frequency of dNOR(+) chromosomes in the carriers of familial translocations and in the control population were not significantly different (X^2_1=0.08, 0.90>p>0.75). There was no significant difference in the incidence of free-lying dNOR chromosomes in the probands (4/25) and the carriers of familial translocations (2/25) (X^2_1=0.76, 0.50>p>0.25).

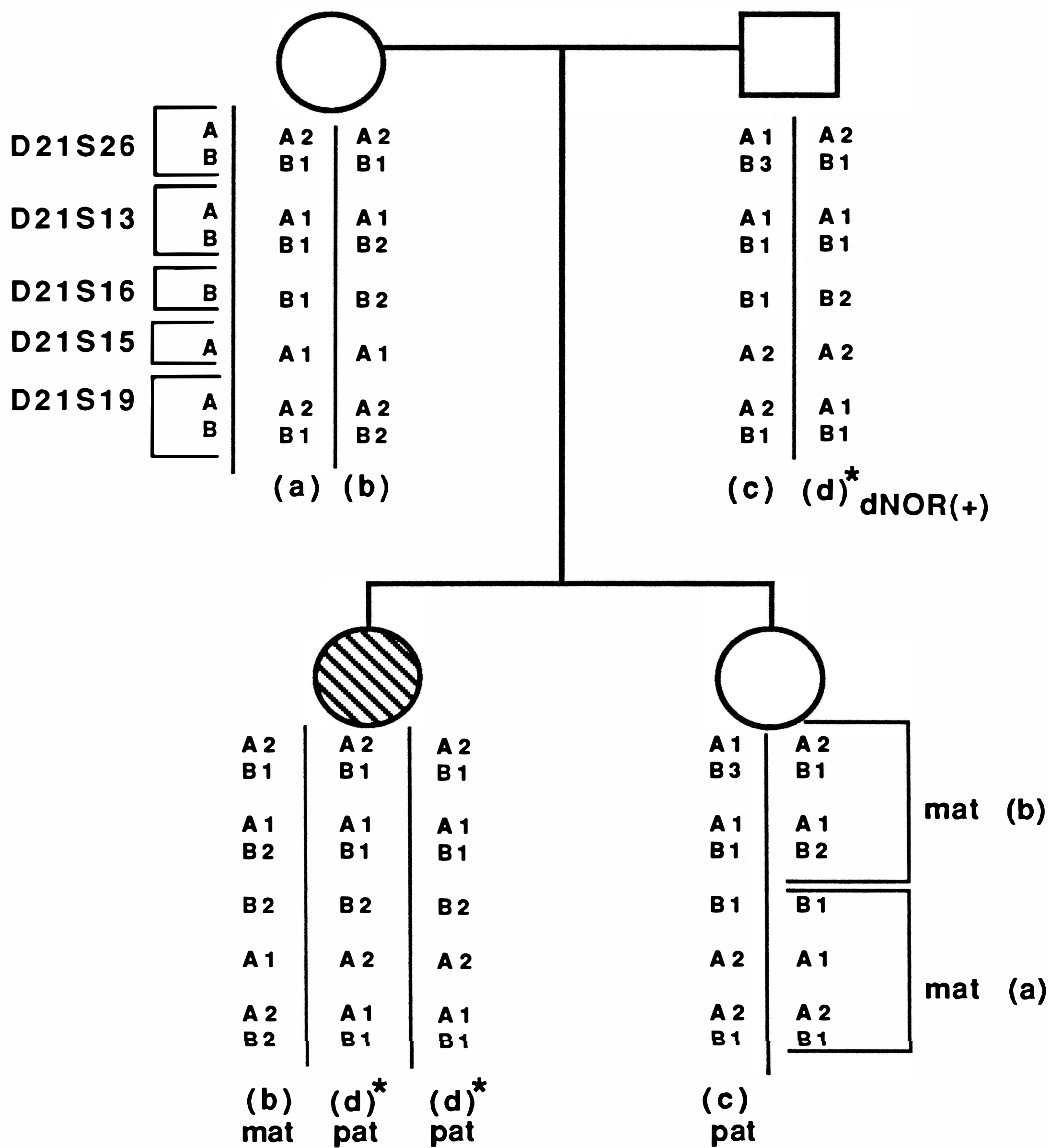
2. Risk assessment

In order to estimate the power of the dNOR variant in predisposing individuals to form de novo acrocentric rearrangements, the absolute and relative risks were estimated for individuals who were dNOR (+) (Table 29). Although the de novo rearrangements in this study appeared to be heterogeneous (i.e. isochromosomes, Robertsonian translocations) and since this was the first study to show a true difference between these rearrangements, no values for the incidence of each type of rearrangement were available. Therefore, the estimated absolute risk was a pooled risk for all Robertsonian-type rearrangements. The absolute risk to individuals who were dNOR(+) for having a child with a de novo Robertsonian-type rearrangement was estimated assuming 1) an incidence of de novo Robertsonian translocations in liveborn infants of 1/25,000 (Hook, 1981); 2) an incidence of individuals who were dNOR(+) among parents who have had a child with a de novo Robertsonian-type rearrangement of 11/25

Table 28. Distribution of dNOR variant chromosome

Family	rea	origin	dNOR	origin	Inheritance by proband
1	i(21q)	paternal	15	paternal	not inherited
			21	paternal	in rearrangement (by RFLP)
2	rob(13q14q)	maternal	15	maternal	free-lying chromosome
3	rob(14q21q)	paternal	21	paternal	free-lying chromosome
5	rea(21q21q)	paternal	21	paternal	unknown
6	rea(21q21q)	paternal	21	paternal	unknown
			22	maternal	free-lying chromosome
8	dic(15;15)(p11;p11)	paternal	21	paternal	not inherited
9	rob(13q14q)	paternal	22	paternal	not inherited
11	rob(13q15q)	maternal	13	maternal	unknown
13	rea(21q21q)	maternal	21	maternal	unknown
15	rob(14q21q)	maternal	15	maternal	not inherited
24	rob(14q21q)	maternal	15	maternal	free-lying chromosome

Fig. 12. Haplotype for family 1 determined by RFLP analysis. The proband has an idic(21q) determined to have originated from the father's dNOR(+) chromosome 21 (d). Her normal sibling did not inherit the dNOR(+) variant and had the alternative paternal haplotype (c).



D21S26
D21S13
D21S16
D21S15
D21S19
A2 B1 A1 B1 B1 A1 A2 B1
A2 B1 A1 B2 B2 A1 A2 B2
(a) (b)
A1 B3 A1 B1 B1 A2 A2 B1
A2 B1 A1 B1 B2 A2 A1 B1
(c) (d)* dNOR(+)
(b) mat
(d)* pat
(d)* pat
(c) pat
mat (b)
mat (a)

Table 29. Risk estimates for individuals who were dNOR(+) (adapted from Jackson-Cook, 1985)

dNOR Phenotype	Frequency of Offspring	
	Robertsonian	non-Robertsonian
dNOR(+)	A. 11/25[a] X 1/25,000[b]	B. 5/50[c] X 24,999/25,000[d]
dNOR(-)	C. 14/25[e] X 1/25,000[b]	D. 45/50[f] X 24,999/25,000[d]

Absolute Risk		
Individual	Formula	Risk
dNOR(+)	A/A+B	0.0176%
dNOR(-)	C/C+D	0.0025%
Relative Risk	A/A+B / C/C+D	7-fold increase

[a] Incidence of dNOR(+) individuals among parents of origin.

[b] Incidence of *de novo* Robertsonian translocations among liveborn infants (Hook, 1981).

[c] Incidence of dNOR(+) individuals in general population (obtained from control subjects).

[d] Incidence of liveborn infants without *de novo* Robertsonian translocations.

[e] Incidence of dNOR(-) individuals among parents of origin.

[f] Incidence of dNOR(-) individuals in general population (obtained from control subjects).

(44%) (obtained from this study); and 3) an incidence of individuals who were dNOR(+) among the general population of 5/50 (obtained from the control population).

The absolute risk to individuals who were dNOR(+) was estimated to be 0.0176% (~1/5,000) and to individuals who were dNOR(-), 0.0025% (~1/40,000). Although these risks were very small, the relative risk for individuals who were dNOR(+), which was estimated from the ratio of the absolute risks for dNOR(+) individuals to dNOR(-) individuals, was found to be seven times higher.

B. Scores

The distributions of the overall NOR scores for each study group are shown in Figure 13. The distributions in the parents of origin, normal spouses, probands, carriers of familial translocations, and controls differed significantly from random. The most common score was 2 for all groups except carriers of familial translocations in which the most common score was 3. The least frequent score in all groups was a score of 4 or greater. There were no significant differences in the frequency of these scores between the study groups.

The distributions for each acrocentric chromosome are shown in Figure 14. In the parents of origin, a score of zero was found most frequently on chromosomes 22 and a score of 4 or greater on chromosomes 21. In the normal spouses, a score of zero was found most frequently on chromosomes 21 and a score of 4 or greater on chromosomes 13. The probands had

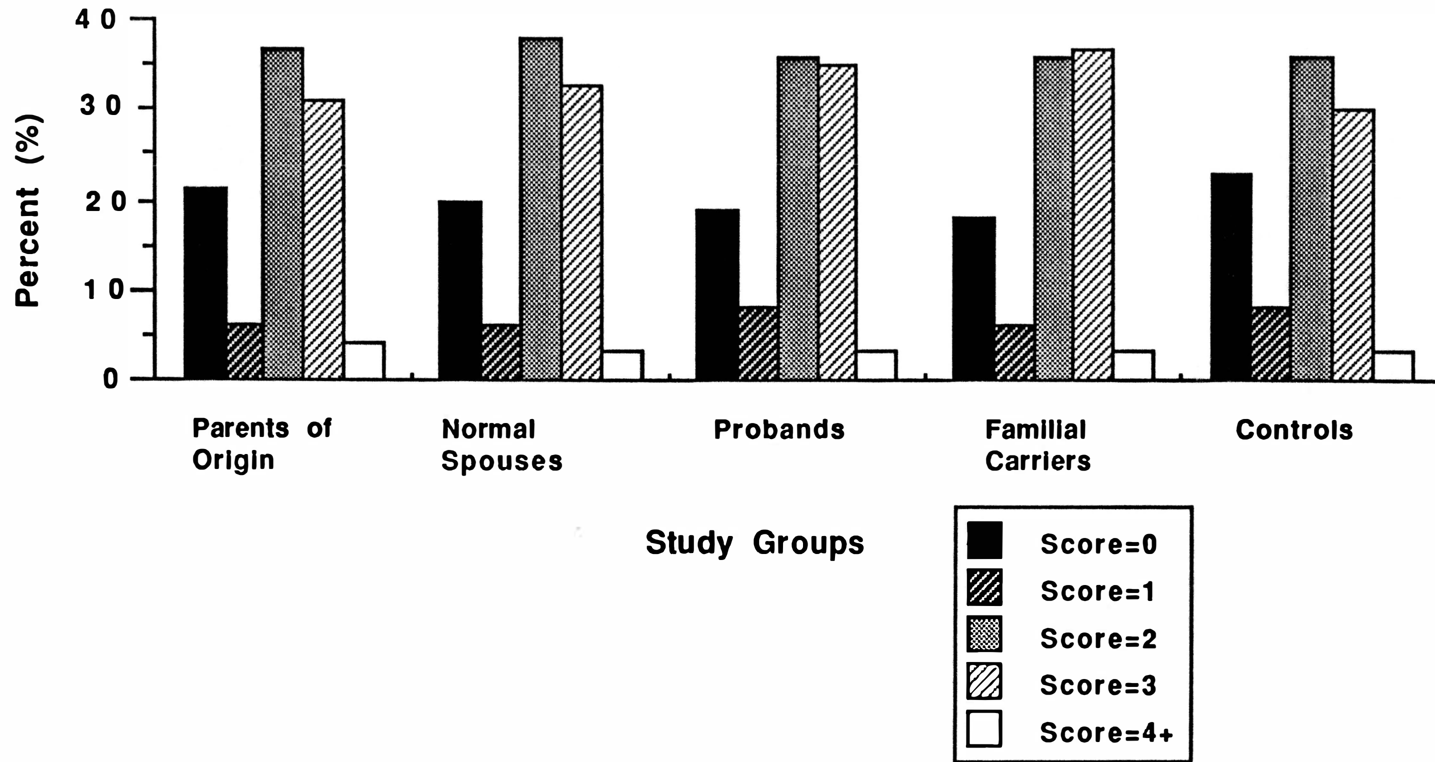


Fig. 13. Distribution of the overall NOR scores in the study groups

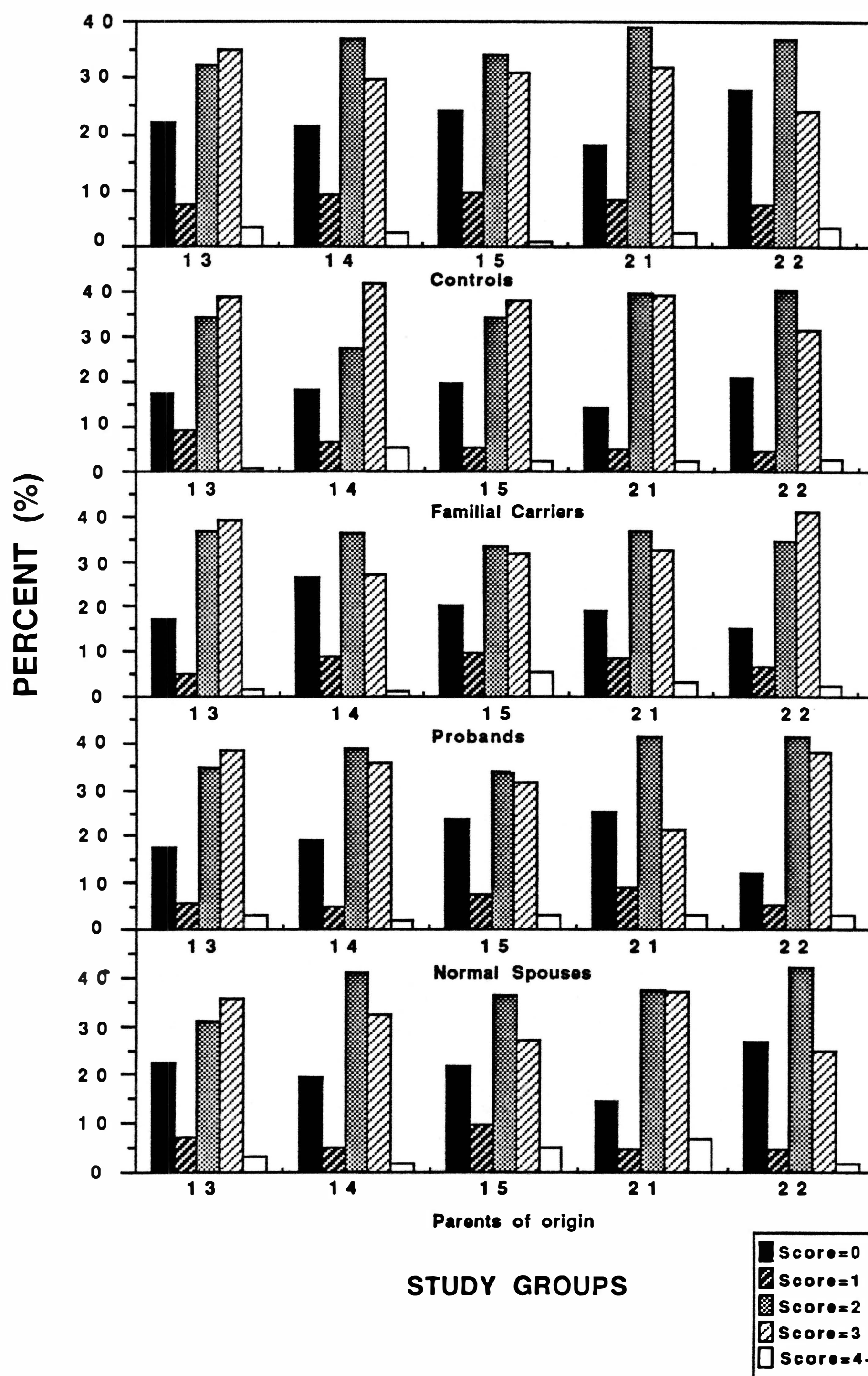


Fig. 14. Distribution of the NOR scores over the acrocentric chromosomes for the study groups.

zeros most often for chromosomes 14 while chromosomes 15 most frequently had a score of 4 or greater. The carriers of familial translocations had the opposite findings; zeros for chromosomes 15 and a score of 4 or greater for chromosomes 14. The controls had a score of zero most frequently on chromosomes 22 and a score of 4 or greater on chromosomes 13 and 22.

The overall mean NOR scores were compared between the parents of origin, normal spouses, probands, carriers of familial translocations and controls (Table 30). Although there was no significant difference between the parents of origin and their normal spouses, these two parental groups had significantly higher mean NOR scores than the control group ($p<0.0001$). The parental groups and the control group had significantly greater mean NOR scores than the probands or carriers of familial translocations ($p<0.0001$). Additionally, the probands had significantly greater NOR scores than familial carriers ($p<0.0001$).

The mean number of silver-positively stained chromosomes were compared between the study groups (Table 30). No significant differences were noted between the parents of origin, their normal spouses or controls. However, the parental groups and the control group had significantly greater mean number of silver-positively stained chromosomes than either the probands or carriers of familial translocations ($p<0.0001$). Additionally, the probands had significantly more silver-positive chromosomes than the

Table 30. Comparison of mean NOR scores and mean number of silver-positive chromosomes in the study groups (Mean±S.E.)

Group	NOR score	Number Ag(+)	Mean NOR score/Ag(+)
Parents of origin (P)	18.67±0.22	7.67±0.06	2.43±0.02
Normal Spouses (N)	18.82±0.24	7.84±0.07	2.40±0.02
Probands (O)	16.93±0.24	6.99±0.09	2.44±0.02
Carriers of familial translocations (F)	15.68±0.18	6.41±0.07	
Controls (C)	17.80±0.16	7.59±0.06	
p-value	$p<0.0001$	$p<0.0001$	$p=0.22$ (ns)
Comparison	P, N > C > O > F	P, N, C > O > F	

carriers of familial translocations ($p<0.0001$).

In order to evaluate compensation of the NORs in the probands, the mean NOR scores per silver-positive acrocentric chromosome were compared between the parents and offspring (Table 30). Although the probands had significantly lower mean NOR scores and significantly fewer silver-positive chromosomes than the parents, the mean NOR score per silver-positive chromosome was not significantly different from the parents ($p=0.22$). Therefore, there was no evidence for compensation of NOR activity in the probands.

The mean NOR scores for each acrocentric chromosome were compared between the study groups (Table 31). When the parents of origin, normal spouses and control individuals were compared, there were no significant differences for the mean NOR scores for chromosomes 13, 14 or 15. However, the parents of origin had a significantly higher mean NOR score for chromosome 21 than the normal spouses or controls ($p<0.0001$). The normal spouses had a significantly higher mean NOR score for chromosome 22 than the parents of origin or the control group ($p<0.0001$).

The probands were compared to their parents. The normal spouses had a significantly higher mean NOR scores for chromosome 13 than the probands ($p=0.02$). Both parental groups had significantly higher mean NOR scores for chromosomes 14 and 21 than the probands ($p<0.0001$). There was no significant difference between the parental groups and the probands for the mean NOR score of chromosome 15 ($p=0.94$).

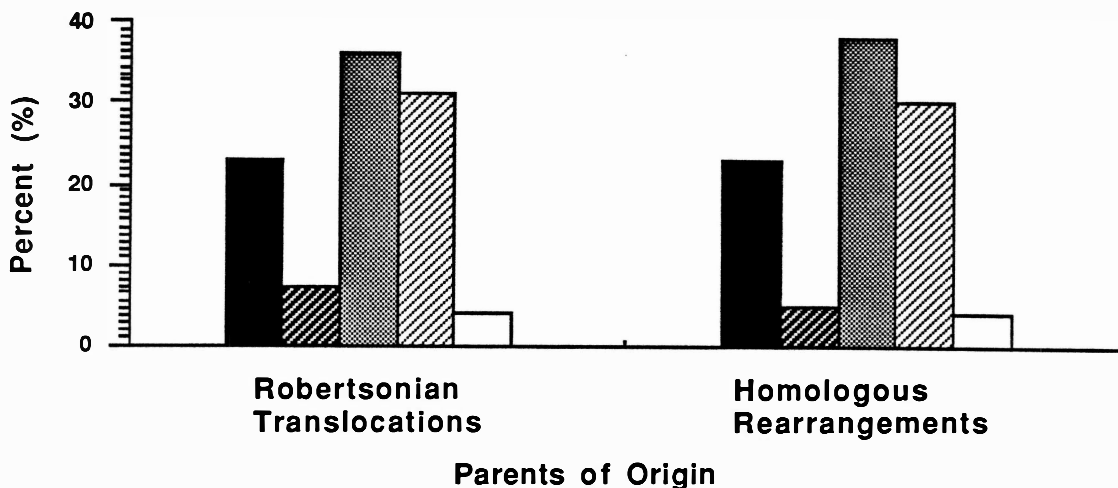
Table 31. Comparison of mean NOR scores distributed over the acrocentric chromosomes in the study groups (Mean±S.E.)

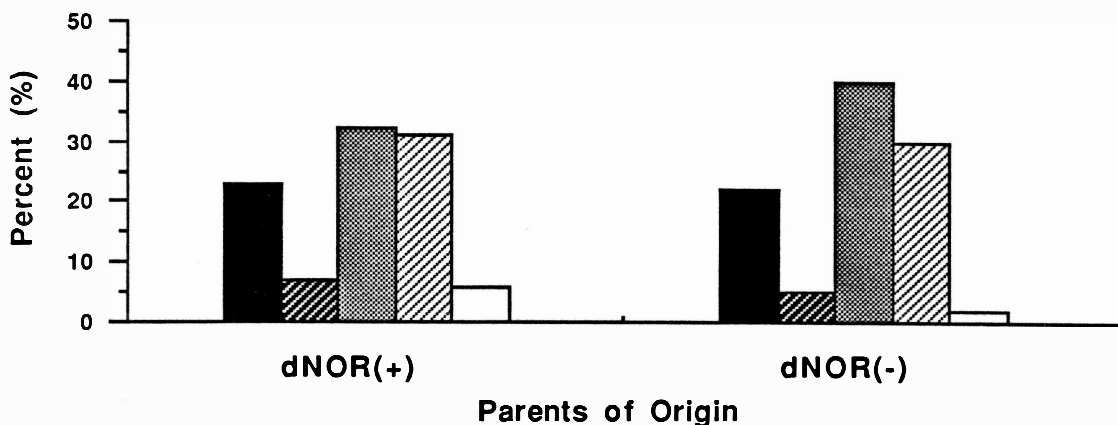
	Chromosomes				
Group	13	14	15	21	22
Parents of origin (P)	3.76±0.11	3.77±0.10	3.61±0.10	4.29±0.11	3.34±0.11
Normal Spouses (N)	4.02±0.10	3.38±0.10	3.56±0.11	3.27±0.10	4.15±0.10
Probands (O)	3.60±0.11	2.73±0.11	3.60±0.12	2.90±0.11	4.07±0.11
Carriers of familial (F) translocations	2.91±0.11	2.28±0.10	3.84±0.11	3.17±0.11	3.48±0.10
Controls (C)	3.76±0.08	3.56±0.07	3.38±0.07	3.79±0.07	3.30±0.08
Comparisons					
P, N, C	p=0.09 (ns)	p=0.06 (ns)	p=0.15 (ns)	p<0.0001 P > C > N	p<0.0001 N > P, C
P, N, O	p=0.02 N > O	p<0.0001 P, N > O	p=0.94 (ns)	p<0.0001 P > N > O	p<0.0001 N, O > P
O, F	p<0.0001 O > F	p<0.0001 O > F	p=0.06 (ns)	p=0.07 (ns)	p<0.0001 O > F

Interestingly, the probands had a greater mean NOR score for chromosome 22 than the parents of origin which likely resulted from the scores noted for chromosome 22 in the non-contributing parent (normal spouses). The probands who carry de novo rearrangements had significantly greater mean NOR scores than the carriers of familial translocations for chromosomes 13, 14 and 22 ($p<0.0001$). There was no significant difference between these groups for chromosomes 15 and 21.

Since the parents in whom the de novo rearrangement originated contributed a heterogeneous population of rearrangements, these parents were divided into several groups: 1) those parents who contributed "true" Robertsonian translocations (rob) and those who contributed homologous rearrangements (hom); 2) those parents who were dNOR(+) and those who were dNOR(-); and 3) those parents who contributed Robertsonian translocations and were dNOR(+) [rob(+)] or dNOR(-) [rob(-)] and those who contributed homologous rearrangements and were dNOR(+) [hom(+)] or dNOR(-) [hom(-)]. Henceforth, the parents of origin will be referred to as "parents" with the appropriate designation [i.e. dNOR(+), dNOR(-), rob, hom].

The distributions of the NOR scores for each acrocentric chromosome are shown in Figures 15 and 16. The distributions of the overall NOR scores differed significantly from random in all groups. The most frequently occurring overall NOR score was 2 in all groups except for the rob(+) parents in





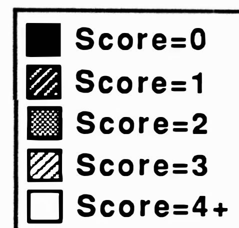


Fig. 15. Distribution of the overall NOR scores in the parents of origin. Shown are the parents of origin who contributed Robertsonian translocations or homologous rearrangements (top) and those who were dNOR(+) or dNOR(-) (bottom).

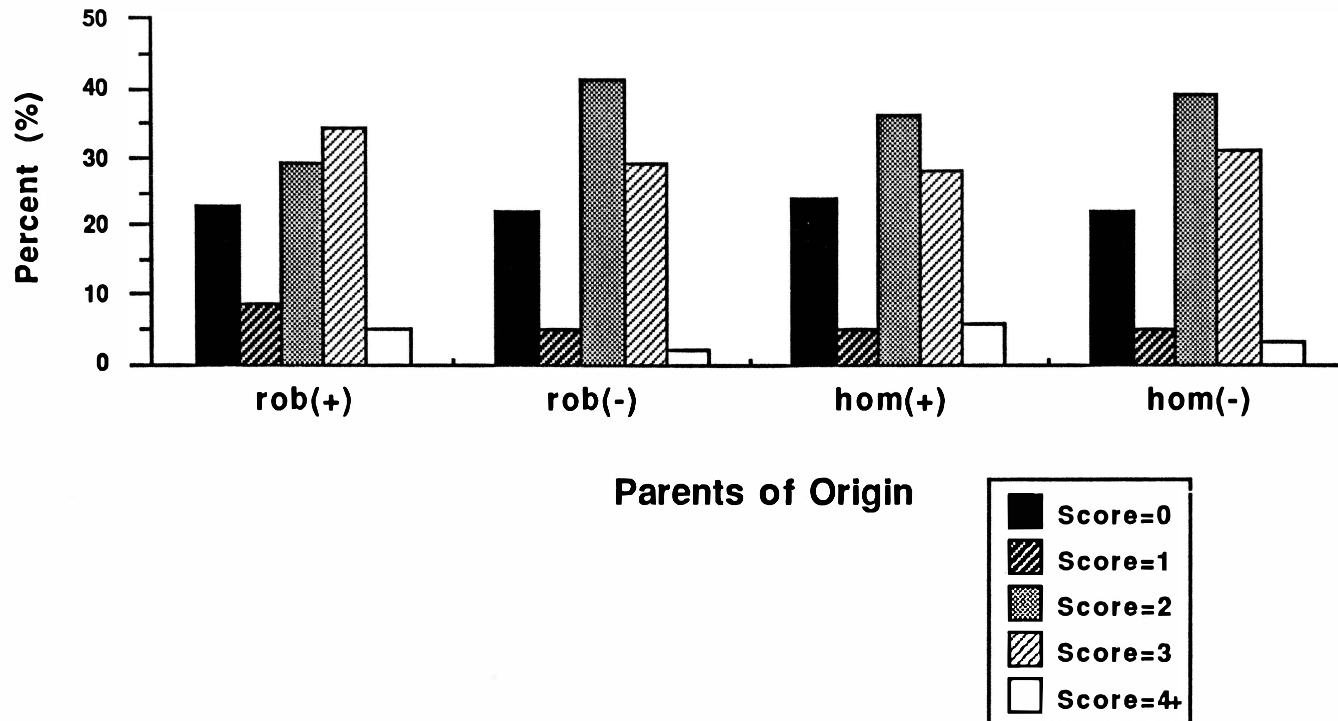


Fig. 16. Distribution of the overall NOR scores in the parents of origin. The parents were divided accordingly: rob(+) = parents who were dNOR(+) and contributed Robertsonian translocations to their offspring, rob(-) = parents who were dNOR(-) and contributed Robertsonian translocations, hom(+) = parents who were dNOR(+) and contributed homologous rearrangements and hom(-) = parents who were dNOR(-) and contributed homologous rearrangements to their offspring.

which the most frequently occurring score was 3. The least frequent score was 4 or greater in all groups except for the hom(+) parents in which the least frequently occurring score was 1. There were no significant differences in the frequency of these scores between the parents of origin.

The distribution of the NOR scores was examined for each chromosome (Fig. 17-19). In the rob, hom, dNOR(+) and dNOR(-) parents, a score of zero was found most frequently for chromosome 22. A score of 4 or greater was found most frequently for chromosome 15 in the rob and dNOR(-) parents and chromosome 21 in the hom and dNOR(+) parents (Fig. 17 and 18). However, a score of zero was found most frequently on chromosome 14 for the rob(+) and hom(-) parents; chromosomes 15 and 22 for the hom(+) parents; and chromosomes 13 and 22 for the rob(-) parents (Fig. 19). A score of 4 or greater was found most frequently on chromosomes 13, 21 and 22 for the rob(+) parents; chromosome 21 for the hom(+) parents; chromosome 15 for the hom(-) parents; and chromosome 14 for the rob(-) parents (Fig. 19).

The overall mean NOR scores and mean number of silver-positively stained chromosomes were compared between the parents of origin (Table 32). There were no significant differences noted between the rob and hom parents; the dNOR(+) and dNOR(-) parents; or the rob(+), rob(-), hom(+), and hom(-) parents in either the mean NOR scores or the mean number of silver-positive chromosomes.

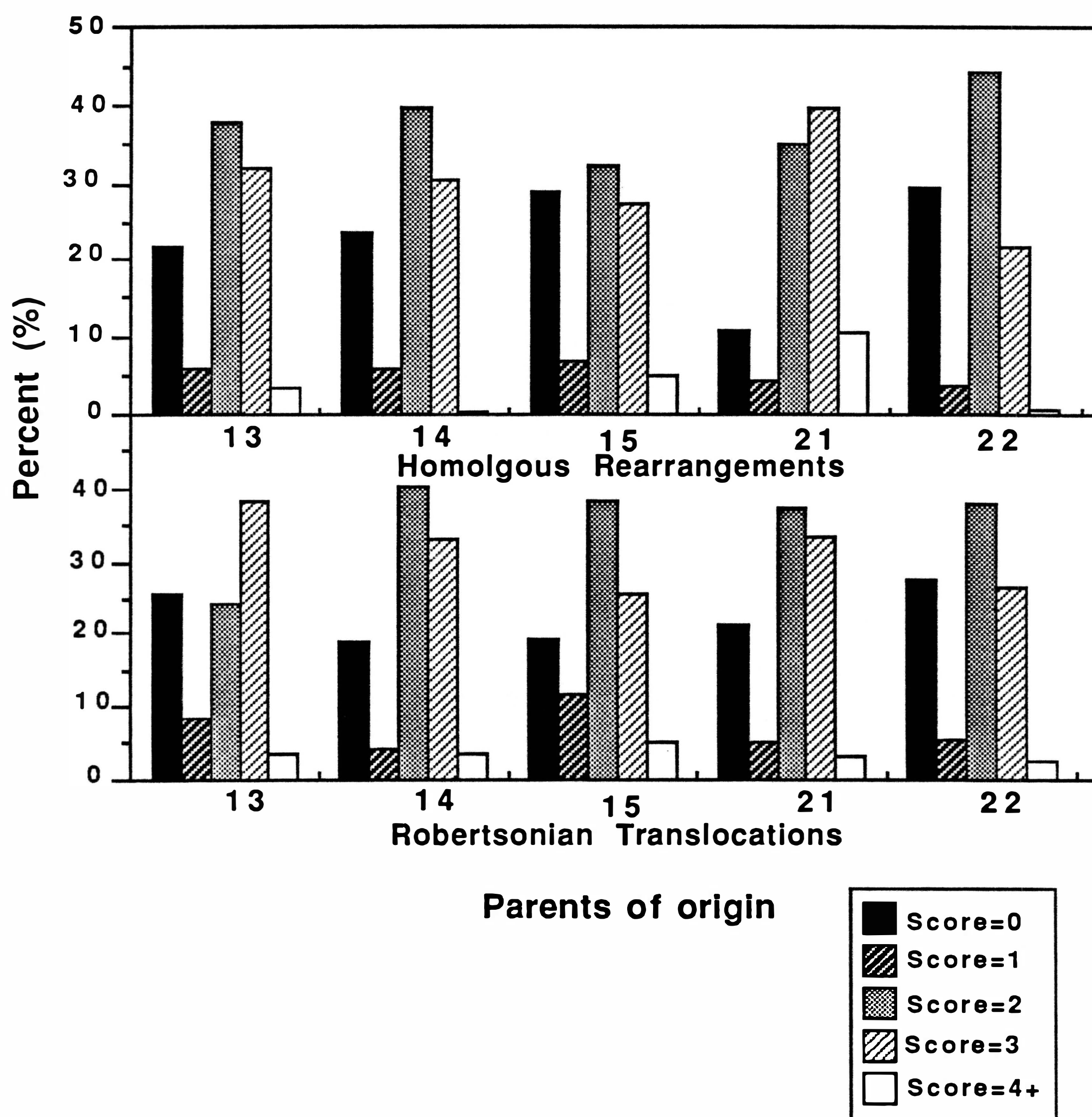


Fig. 17. Distribution of the NOR scores over the acrocentric chromosomes for the parents of origin. Shown are those parents who contributed homologous rearrangements (top) or Robertsonian translocations (bottom) to their offspring.

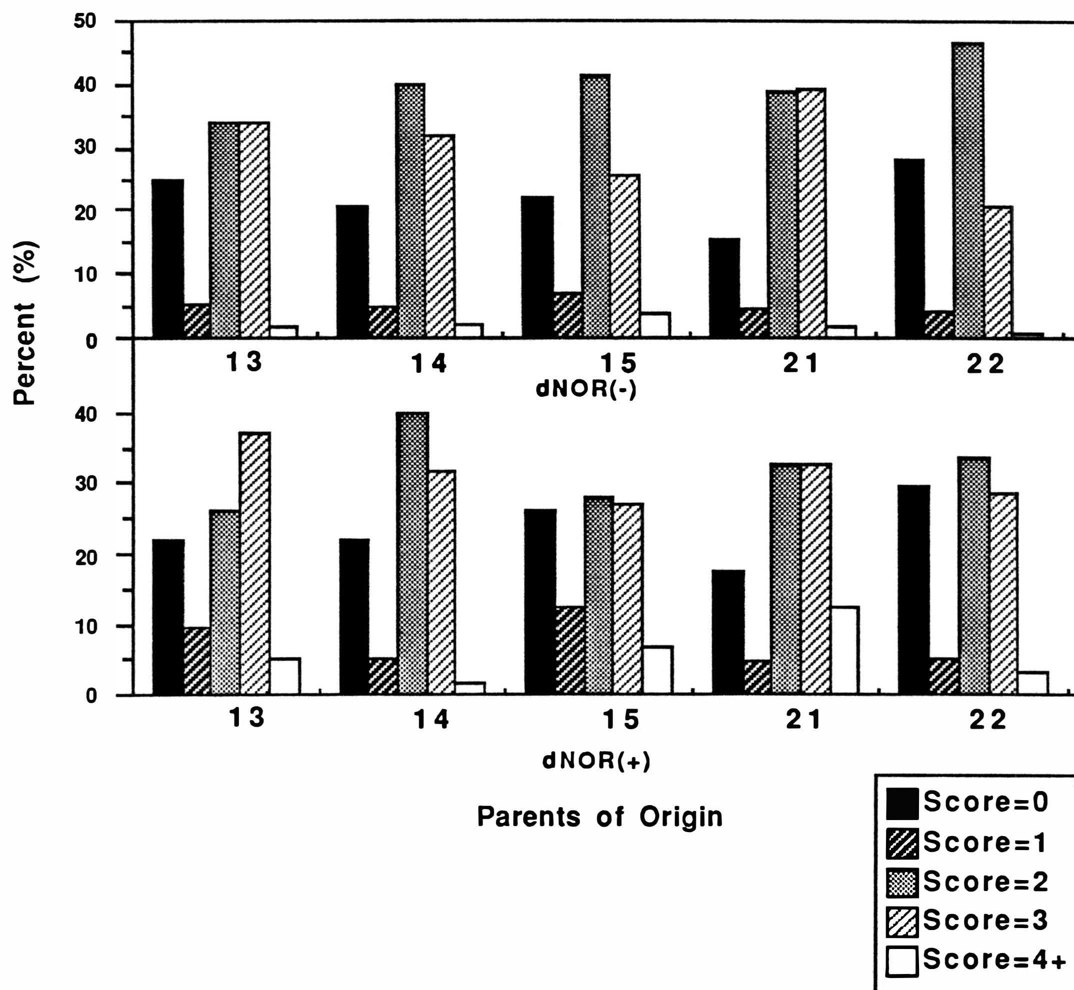


Fig. 18. Distribution of the NOR scores over the acrocentric Chromosomes for the parents of origin. Shown are those parents who were dNOR(-) (top) and dNOR(+) (bottom).

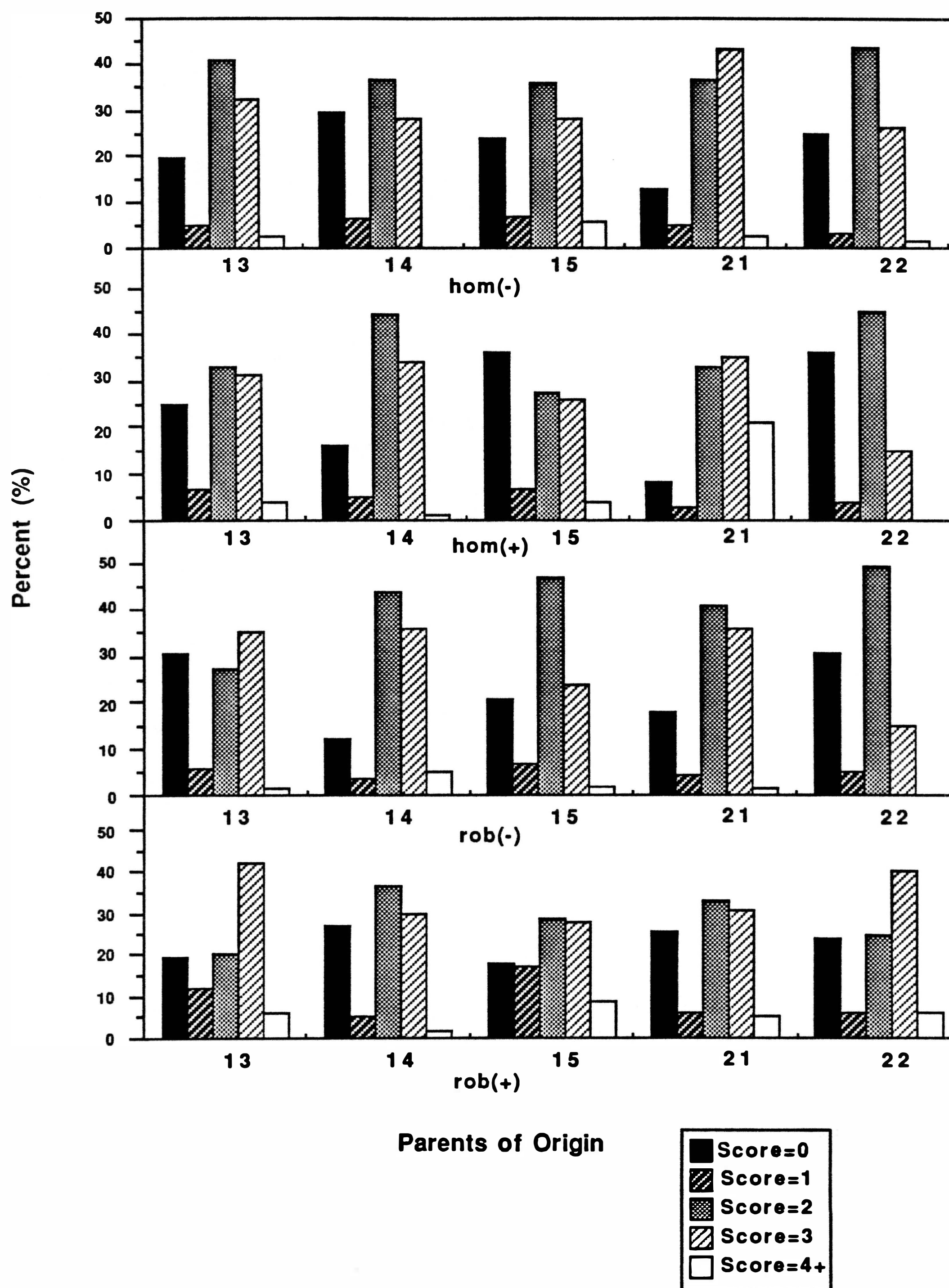


Fig. 19. Distribution of the NOR scores over the acrocentric chromosomes for the parents of origin.

Table 32. Comparison of mean NOR scores and mean number of silver-positive chromosomes in the parents of origin (Mean±S.E.)

	rob	hom	p-value	dNOR(+)	dNOR(-)	p-value
NOR score	18.71±0.32	18.63±0.32	p=0.84 (ns)	18.87±0.30	18.51±0.32	p=0.40 (ns)
Ag(+)	7.74±0.10	7.61±0.11	p=0.38 (ns)	7.54±0.09	7.78±0.11	p=0.11 (ns)

Table 32. continued

	rob(+)	rob(-)	hom(+)	hom(-)	p-value
NOR score	19.25±0.40	18.26±0.47	18.42±0.45	18.77±0.45	p=0.40 (ns)
Ag(+)	7.73±0.12	7.74±0.15	7.32±0.14	7.81±0.15	p=0.10 (ns)

The mean NOR scores for each acrocentric chromosome were compared between the parents of origin (Table 33). No significant differences were noted between the rob and hom parents for the mean NOR scores for chromosomes 13, 14, 15 or 22. However, for chromosome 21, the hom parents had a significantly greater mean NOR score than the rob parents ($p<0.0001$). No significant differences were noted between the dNOR(+) and dNOR(-) parents for mean NOR scores of any acrocentric chromosome. When the hom(+) and hom(-) parents were compared, there was no significant difference in mean NOR score of chromosome 13. However, the hom(+) parents had significantly higher mean NOR scores than the hom(-) parents for chromosomes 14 and 21 and the hom(-) parents had significantly greater mean NOR scores than the hom(+) parents for chromosomes 15 and 22. When the rob(+) and rob(-) parents were compared, there was no significant difference for chromosomes 15 and 21. However, the rob(+) parents had significantly greater mean NOR scores than the rob(-) parents for chromosomes 13 and 22 while the rob(-) parents had significantly greater mean NOR scores than the rob(+) parents for chromosome 14.

VI. Satellite associations

Since satellite associations may contribute to the formation of Robertsonian-type translocations, the study groups were compared with respect to: 1) mean number of satellite associations; 2) mean number of chromosomes which participated in satellite associations; and 3) mean number of

Table 33. Comparison of mean NOR scores distributed over the acrocentric chromosomes in the parents of origin (mean±S.E.)

Group	Chromosomes 13	14	15	21	22
rob	3.74±0.15	3.95±0.15	3.74±0.15	3.84±0.14	3.47±0.15
hom	3.78±0.18	3.58±0.14	3.47±0.13	4.78±0.15	3.21±0.16
dNOR(+)	3.90±0.17	3.72±0.17	3.56±0.18	4.45±0.18	3.47±0.18
dNOR(-)	3.65±0.15	3.81±0.13	3.65±0.12	4.16±0.13	3.24±0.14
rob(+)	4.12±0.20	3.47±0.24	3.93±0.28	3.68±0.23	4.05±0.24
rob(-)	3.41±0.21	4.36±0.17	3.59±0.15	3.97±0.19	2.97±0.16
hom(+)	3.64±0.29	4.02±0.23	3.12±0.18	5.36±0.24	2.78±0.23
hom(-)	3.89±0.22	3.26±0.16	3.71±0.18	4.36±0.17	3.51±0.21
Comparisons					
rob vs hom	p=0.84	p=0.06	p=0.17	p<0.0001 hom > rob	p=0.24
dNOR(+) vs dNOR(-)	p=0.28	p=0.69	p=0.66	p=0.19	p=0.30
rob(+) vs rob(-)	p=0.017 (+)>(-)	p=0.002 (-)>(+)	p=0.25	p=0.31	p=0.0002 (+)>(-)
hom(+) vs hom(-)	p=0.49	p=0.0068 (+)>(-)	p=0.027 (-)>(+)	p=0.0006 (+)>(-)	p=0.02 (-)>(+)

chromosomes per satellite association (Table 34). There were no significant differences between the parents of origin, their normal spouses and the control group for mean number of satellite associations, chromosomes in satellite associations or chromosomes per satellite association. Likewise, there were no significant differences noted between the two parental groups and the probands for mean number of satellite associations or mean number of chromosomes per satellite association. However, as expected, since the probands have fewer chromosomes available for participation in satellite associations, the parental groups had significantly more chromosomes that participated in satellite associations than the probands (p<0.0001). Although there was no significant difference in the mean number of chromosomes per satellite association, the mean number of satellite associations and mean number of chromosomes in satellite associations differed between the probands and the carriers of familial translocations (p<0.0001 O>F).

The involvement in satellite associations of each acrocentric chromosome was evaluated for each group through the calculation of a mean association index (AI). The mean association indexes were then compared between groups (Table 35). There were no significant differences between the parents of origin, their normal spouses and the control group for the mean AI of chromosomes 13, 14, 15 or 22. The control group was found to have a significantly greater mean AI for chromosome 21 than the normal spouses (p=0.008).

Table 34. Mean satellite associations (SA), mean number of chromosomes participated in satellite associations and mean number of chromosomes per satellite association in the study groups (Mean±S.E.)

Group	SA	Chrom	Chrom/SA
Parents of origin (P)	15.4±0.64	34.2±1.45	2.22±0.02
Normal spouses (N)	15.8±0.66	35.0±1.52	2.21±0.03
Probands (O)	13.6±0.65	29.2±1.50	2.15±0.04
Carriers of familial translocations (F)	11.2±0.63	24.8±1.49	2.21±0.03
Controls (C)	16.3±0.55	36.22±1.25	2.23±0.03
p-value	$p<0.0001$ P, N, O, C > F	$p<0.0001$ P, N, C > O > F	$p=0.50$ (ns)
	$p=0.58$ (ns) P = N = C	$p=0.58$ (ns) P = N = C	

Table 35. Comparison of mean association indexes (AI) for each acrocentric chromosome between the study groups

	Chromosomes				
Group	13	14	15	21	22
Parents of origin (P)	0.40±0.03	0.45±0.03	0.42±0.03	0.47±0.04	0.41±0.03
Normal Spouses (N)	0.44±0.04	0.44±0.03	0.45±0.03	0.41±0.03	0.46±0.04
Probands (O)	0.37±0.03	0.38±0.04	0.37±0.03	0.50±0.04	0.43±0.03
Carriers of familial (F) translocations	0.35±0.04	0.39±0.04	0.37±0.03	0.41±0.04	0.41±0.04
Controls (C)	0.47±0.02	0.47±0.03	0.47±0.03	0.55±0.03	0.46±0.03
<u>Comparisons</u>					
P, N, C (ns)	p=0.22 (ns)	p=0.71 (ns)	p=0.52 (ns)	p=0.006 C > N	p = 0.41
P, N, O	p=0.38 (ns)	p=0.32 (ns)	p=0.23 (ns)	p=0.24 (ns)	p=0.48 (ns)
O, F (ns)	p=0.68 (ns)	p=0.88 (ns)	p=0.99 (ns)	p= 0.13 (ns)	p= 0.82

No significant differences were noted between the parental groups and the probands for any acrocentric AI. Likewise, no significant differences were noted for any AI between the probands and the carriers of familial translocations. These findings were expected since the AI calculation accounted for the number of specific chromosomes available for satellite associations, thus accounting for the loss of available chromosomes because of the rearrangements.

The pairwise satellite associations were examined for each group (Table 36). In every study group, the distribution of pairwise satellite associations differed from random. In the parents of origin, there was an increased frequency of 21/21 associations. In the normal spouses, there was a higher rate of 22/22 associations. In the probands, as expected because of the loss of particular chromosomes to the rearrangements, there were decreases in some satellite associations. All associations of chromosome 14 were decreased. Interestingly, there was an increased frequency of 21/22 satellite associations. The carriers of familial translocations had increased 22/22 associations. The control group had increased 21/21 and 21/22 associations.

In order to examine differences between the groups, the mean pairwise satellite associations were compared (Table 37). There were no significant differences noted in the mean pairwise satellite associations between the parents of origin, their normal spouses and controls for all associations except for 21/22 in which the controls were significantly greater

Table 36. Total pairwise satellite associations in the study groups [observed(expected)]

SA	parents of origin	normal spouses	probands	familial carriers	controls
13/13	8 (12.44)	15 (12.91)	10 (10.38)	3 (8.64)	29 (27.9)
13/14	49 (49.78)	57 (51.64)	36 (41.51)	29 (35.58)	120 (111.5)
13/15	49 (49.78)	53 (51.64)	45 (41.51)	34 (35.58)	112 (111.5)
13/21	42 (49.78)	47 (51.64)	45 (41.51)	24 (35.58)	108 (111.5)
13/22	43 (49.78)	46 (51.64)	39 (41.51)	33 (35.58)	91 (111.5)
14/14	10 (12.44)	13 (12.91)	3 (10.38)	0 (8.64)	24 (27.9)
14/15	47 (49.78)	59 (51.64)	34 (41.51)	28 (35.58)	100 (111.5)
14/21	58 (49.78)	40 (51.64)	28 (41.51)	24 (35.58)	120 (111.5)
14/22	53 (49.78)	50 (51.64)	37 (41.51)	25 (35.58)	93 (111.5)
15/15	15 (12.44)	10 (12.91)	12 (10.38)	12 (8.64)	24 (27.9)
15/21	52 (49.78)	44 (51.64)	31 (41.51)	42 (35.58)	123 (111.5)
15/22	44 (49.78)	47 (51.64)	50 (41.51)	49 (35.58)	92 (111.5)
21/21	29 (12.44)	17 (12.91)	15 (10.38)	14 (8.64)	42 (27.9)
21/22	46 (49.78)	53 (51.64)	64 (41.51)	50 (35.58)	142 (111.5)
22/22	15 (12.44)	30 (12.91)	18 (10.38)	22 (8.64)	34 (27.9)
X^2_{14}=	30.1	31.85	37.46	63.45	31.97
p-value	$0.01 > p > 0.005$	$p < 0.005$	$p < 0.005$	$p < 0.005$	$p < 0.005$

Table 37. Comparison of mean pairwise satellite associations between the study groups (Mean±S.E.)

SA	Parents of origin	normal spouses	controls	p-value
13/13	0.03±0.01	0.06±0.02	0.06±0.01	p=0.25 (ns)
13/14	0.20±0.03	0.23±0.03	0.24±0.02	p=0.47 (ns)
13/15	0.20±0.03	0.21±0.03	0.23±0.02	p=0.72 (ns)
13/21	0.19±0.02	0.17±0.03	0.22±0.02	p=0.32 (ns)
13/22	0.17±0.03	0.18±0.03	0.18±0.02	p=0.94 (ns)
14/14	0.04±0.01	0.05±0.01	0.05±0.01	p=0.81 (ns)
14/15	0.19±0.03	0.24±0.03	0.20±0.02	p=0.50 (ns)
14/21	0.23±0.03	0.16±0.03	0.24±0.02	p=0.08 (ns)
14/22	0.21±0.03	0.20±0.03	0.19±0.02	p=0.76 (ns)
15/15	0.06±0.02	0.04±0.01	0.05±0.01	p=0.58 (ns)
15/21	0.21±0.03	0.18±0.03	0.25±0.02	p=0.15 (ns)
15/22	0.18±0.03	0.19±0.03	0.19±0.02	p=0.95 (ns)
21/21	0.12±0.02	0.07±0.02	0.08±0.01	p=0.08 (ns)
21/22	0.18±0.03	0.21±0.03	0.29±0.03	p=0.03 C>P
22/22	0.06±0.02	0.12±0.02	0.07±0.01	p=0.02 N>P

Table 37 continued

SA	probands	P,N vs O p-value	familial carriers	O vs F p-values
13/13	0.04±0.01	p=0.29 (ns)	0.01±0.01	p=0.05
13/14	0.14±0.03	p=0.10 (ns)	0.12±0.02	p=0.41 (ns)
13/15	0.18±0.03	p=0.73 (ns)	0.14±0.02	p=0.23 (ns)
13/21	0.18±0.03	p=0.87 (ns)	0.10±0.02	p=0.01 O>F
13/22	0.16±0.03	p=0.80 (ns)	0.13±0.02	p=0.51 (ns)
14/14	0.01±0.01	p=0.04 P,N>O	0.00±0.00	p=0.08 (ns)
14/15	0.14±0.02	p=0.04 N>O	0.11±0.02	p=0.47 (ns)
14/21	0.11±0.02	p=0.006 P>O	0.10±0.02	p=0.61 (ns)
14/22	0.14±0.02	p=0.20 (ns)	0.10±0.02	p=0.13 (ns)
15/15	0.05±0.01	p=0.58 (ns)	0.05±0.01	p=0.97 (ns)
15/21	0.12±0.02	p=0.09 (ns)	0.21±0.03	p=0.03 F>O
15/22	0.20±0.03	p=0.82 (ns)	0.20±0.03	p=0.97 (ns)
21/21	0.06±0.02	p=0.02 P>N,O	0.06±0.01	p=0.88 (ns)
21/22	0.26±0.03	p=0.26 (ns)	0.20±0.03	p=0.23 (ns)
22/22	0.07±0.02	p=0.04 N>P	0.09±0.02	p=0.49 (ns)

than the parents of origin (p=0.03) and for 22/22 in which the normal spouses were greater than the controls and the parents of origin (p=0.02). Although not significant, the parents of origin had the greatest frequency of 21/21 satellite associations. In most pairwise satellite associations, there were no significant differences between the parents of origin (P), their normal spouses (N) and the probands (O). However, as expected, there were differences in some satellite associations: 14/14 (p=0.04, P,N>O); 14/15 (p=0.04, N>O); 14/21 (p=0.006, P>O); and 21/21 (p=0.02, P>O). Finally, there were no significant differences noted between the probands (O) and the carriers of familial translocations (F) for most pairwise associations except for 13/21 (p=0.01, O>F) and 15/21 (p=0.03, F>O).

Since the parents of origin may have contributed a heterogeneous group of rearrangements, the group was divided into 1) those parents who contributed "true" Robertsonian translocations (rob) and those who contributed homologous rearrangements (hom); 2) parents of origin who were dNOR(+) and dNOR(-); and 3) a combination of the above rob(+), rob(-), hom(+) and hom(-). Henceforth, the parents of origin will be referred to as "parents" with the appropriate designation. There were no significant differences noted in the mean number of satellite associations, mean number of chromosomes which participated in satellite associations, and mean number of chromosomes per satellite association for any comparison of the parents of origin (Table 38).

Table 38. Mean satellite associations (SA), mean number of chromosomes participated in satellite associations and mean number of chromosomes per satellite association in the parents of origin (Mean±S.E.)

Parents of origin	SA	Chrom	Chrom/SA
rob	15.4±0.94	34.0±2.16	2.21±0.04
hom	15.4±0.91	34.4±2.02	2.23±0.03
p-value	p=0.98 (ns)	p=0.89 (ns)	p=0.62 (ns)
dNOR(+)	15.9±1.10	35.3±2.48	2.22±0.04
dNOR(-)	15.0±0.78	33.4±1.77	2.22±0.03
p-value	p=0.49 (ns)	p=0.52 (ns)	p=0.89 (ns)
rob(+)	15.0±1.69	33.0±3.85	2.20±0.06
rob(-)	15.7±1.08	34.9±2.55	2.22±0.05
hom(+)	17.0±1.30	38.0±2.88	2.24±0.06
hom(-)	14.3±1.13	31.9±2.50	2.23±0.04
p-value	p=0.55 (ns)	p=0.54 (ns)	p=0.96 (ns)

The association indexes for each acrocentric chromosome were calculated and compared between the parents (Table 39). There were no significant differences noted in any of the comparisons except that the dNOR(+) parents had a significantly higher mean AI for chromosome 14 than the dNOR(-) parents (p=0.046).

The pairwise satellite association distributions were examined (Table 40) and did not differ significantly from random for the dNOR(+) parents. However, the distributions were significantly different from random for the dNOR(-) parents (0.025>p>0.01) and the hom parents (p=0.025) and was borderline significant for the rob parents (p=0.05). The pairwise distributions did not differ from random in the rob(+) and hom(-) parents. However, the distributions were significantly different from random in the rob(-) (p<0.005) and hom(+) parents (0.05>p>0.025).

The mean pairwise distributions were compared between the parents of origin (Table 41). No significant differences were noted between the dNOR(+) and dNOR(-) parents. Although no rea(13q13q) were observed among the probands, the only significant difference found between the rob and hom parents was in the 13/13 pairwise satellite association (p=0.02, hom>rob). No significant differences were noted in any pairwise satellite association between the rob(+) and rob(-) parents. Although not statistically significant, the rob(-) parents had ~2.5X higher 21/21 satellite associations than the rob(+) parents. The only significant differences noted

Table 39. Comparison of mean association indexes (AI) for each acrocentric chromosome between the parents of origin

Parents of origin	Chromosomes 13	14	15	21	22
rob	0.39±0.05	0.47±0.05	0.47±0.05	0.45±0.05	0.45±0.04
hom	0.41±0.04	0.43±0.05	0.37±0.04	0.50±0.05	0.36±0.04
p-value	p=0.75 (ns)	p=0.65 (ns)	p=0.13 (ns)	p=0.52 (ns)	p=0.12 (ns)
dNOR(+)	0.44±0.05	0.53±0.06	0.44±0.05	0.47±0.06	0.41±0.04
dNOR(-)	0.38±0.04	0.39±0.04	0.40±0.04	0.47±0.04	0.41±0.04
p-value	p=0.35 (ns)	p=0.046	p=0.56 (ns)	p=0.99 (ns)	p=0.99 (ns)
rob(+)	0.46±0.08	0.54±0.08	0.45±0.07	0.41±0.07	0.41±0.06
rob(-)	0.34±0.05	0.40±0.06	0.48±0.07	0.49±0.06	0.49±0.06
hom(+)	0.41±0.07	0.51±0.08	0.43±0.07	0.55±0.09	0.41±0.07
hom(-)	0.41±0.06	0.38±0.06	0.33±0.05	0.46±0.07	0.32±0.05
p-value	p=0.59 (ns)	p=0.25 (ns)	p=0.31 (ns)	p=0.61 (ns)	p=0.21 (ns)

Table 40. Total pairwise satellite associations in parents of origin [observed(expected)]

SA	rob	hom	dNOR(+)	dNOR(-)
13/13	1 (6.62)	7 (5.84)	2 (5.75)	6 (6.73)
13/14	24 (26.48)	25 (23.37)	27 (23.0)	22 (26.93)
13/15	29 (26.48)	20 (23.37)	23 (23.0)	26 (26.93)
13/21	17 (26.48)	25 (23.37)	16 (23.0)	26 (26.93)
13/22	25 (26.48)	18 (23.37)	22 (23.0)	21 (26.93)
14/14	6 (6.62)	4 (5.84)	7 (5.75)	3 (6.73)
14/15	29 (26.48)	18 (23.37)	23 (23.0)	24 (26.93)
14/21	28 (26.48)	30 (23.37)	30 (23.0)	28 (26.93)
14/22	26 (26.48)	27 (23.37)	25 (23.0)	28 (26.93)
15/15	7 (6.62)	8 (5.84)	9 (5.75)	6 (6.73)
15/21	25 (26.48)	27 (23.37)	22 (23.0)	30 (26.93)
15/22	29 (26.48)	15 (23.37)	18 (23.0)	26 (26.93)
21/21	16 (6.62)	15 (5.84)	12 (5.75)	19 (6.73)
21/22	27 (26.48)	18 (23.37)	17 (23.0)	29 (26.93)
22/22	9 (6.62)	6 (5.84)	6 (5.75)	9 (6.73)
$X^2_{14}=$	23.61	26.40	19.22	28.58
p-value	$p=0.05$	$p=0.025$	$0.25>p>0.10$	$0.025>p>0.010$

Table 40. continued

SA	rob(+)	rob(-)	hom(+)	hom(-)
13/13	0 (3.0)	1 (3.62)	2 (2.67)	5 (3.11)
13/14	15 (12.0)	9 (14.49)	10 (10.67)	13 (12.44)
13/15	14 (12.0)	15 (14.49)	9 (10.67)	11 (12.44)
13/21	7 (12.0)	10 (14.49)	9 (10.67)	16 (12.44)
13/22	15 (12.0)	10 (14.49)	7 (10.67)	11 (12.44)
14/14	3 (3.0)	3 (3.62)	4 (2.67)	0 (3.11)
14/15	16 (12.0)	13 (14.49)	7 (10.67)	11 (12.44)
14/21	16 (12.0)	12 (14.49)	14 (10.67)	16 (12.44)
14/22	11 (12.0)	15 (14.49)	14 (10.67)	13 (12.44)
15/15	3 (3.0)	4 (3.62)	6 (2.67)	2 (3.11)
15/21	9 (12.0)	16 (14.49)	13 (10.67)	14 (12.44)
15/22	11 (12.0)	18 (14.49)	7 (10.67)	8 (12.44)
21/21	4 (3.0)	12 (3.62)	8 (2.67)	7 (3.11)
21/22	9 (12.0)	18 (14.49)	8 (10.67)	11 (12.44)
22/22	2 (3.0)	7 (3.62)	4 (2.67)	2 (3.11)
$X^2_{14}=$	11.92	31.93	23.89	14.45
p-value	$0.75>p>0.50$	$p<0.005$	$0.05>p>0.025$	$0.5>p>0.25$

Table 41. Comparison of mean pairwise satellite associations between the parents of origin (Mean±S.E.)

SA	rob	hom	p-value	dNOR(+)	dNOR(-)	p-value
13/13	0.01±0.01	0.06±0.02	p=0.02	0.02±0.01	0.04±0.02	p=0.28 (ns)
13/14	0.19±0.04	0.21±0.04	p=0.69 (ns)	0.25±0.05	0.16±0.03	p=0.11 (ns)
13/15	0.22±0.04	0.17±0.04	p=0.31 (ns)	0.21±0.05	0.19±0.04	p=0.66 (ns)
13/21	0.13±0.03	0.21±0.04	p=0.12 (ns)	0.15±0.03	0.19±0.03	p=0.43 (ns)
13/22	0.19±0.04	0.15±0.03	p=0.39 (ns)	0.20±0.04	0.15±0.03	p=0.31 (ns)
14/14	0.05±0.02	0.03±0.02	p=0.59 (ns)	0.06±0.02	0.02±0.01	p=0.09 (ns)
14/15	0.22±0.04	0.15±0.03	p=0.17 (ns)	0.21±0.04	0.17±0.04	p=0.47 (ns)
14/21	0.22±0.04	0.25±0.05	p=0.61 (ns)	0.27±0.05	0.20±0.04	p=0.24 (ns)
14/22	0.20±0.04	0.23±0.04	p=0.67 (ns)	0.23±0.04	0.20±0.04	p=0.60 (ns)
15/15	0.05±0.02	0.07±0.02	p=0.68 (ns)	0.08±0.03	0.04±0.02	p=0.19 (ns)
15/21	0.19±0.04	0.23±0.05	p=0.62 (ns)	0.20±0.05	0.21±0.04	p=0.84 (ns)
15/22	0.22±0.04	0.13±0.03	p=0.05	0.17±0.04	0.19±0.04	p=0.69 (ns)
21/21	0.12±0.03	0.13±0.03	p=0.98 (ns)	0.11±0.03	0.14±0.03	p=0.56 (ns)
21/22	0.21±0.04	0.16±0.04	p=0.39 (ns)	0.21±0.04	0.16±0.04	p=0.39 (ns)
22/22	0.07±0.02	0.05±0.02	p=0.51 (ns)	0.06±0.02	0.06±0.02	p=0.76 (ns)

Table 41. continued

SA	rob(+)	rob(-)	hom(+)	hom(-)	p-value
13/13	0.00±0.00	0.01±0.01	0.04±0.03	0.07±0.03	p=0.47 (ns)
13/14	0.25±0.07	0.13±0.04	0.24±0.07	0.19±0.05	p=0.53 (ns)
13/15	0.23±0.06	0.21±0.06	0.18±0.07	0.16±0.04	p=0.76 (ns)
13/21	0.14±0.04	0.12±0.04	0.18±0.05	0.23±0.05	p=0.54 (ns)
13/22	0.25±0.06	0.14±0.04	0.14±0.05	0.16±0.05	p=0.81 (ns)
14/14	0.05±0.03	0.04±0.02	0.08±0.04	0.00±0.00	p=0.02
14/15	0.27±0.06	0.19±0.05	0.14±0.05	0.16±0.05	p=0.81 (ns)
14/21	0.27±0.07	0.17±0.05	0.28±0.08	0.23±0.06	p=0.61 (ns)
14/22	0.19±0.05	0.21±0.05	0.28±0.08	0.19±0.05	p=0.27 (ns)
15/15	0.05±0.03	0.06±0.03	0.12±0.05	0.03±0.02	p=0.05
15/21	0.15±0.05	0.23±0.06	0.26±0.08	0.20±0.06	p=0.53 (ns)
15/22	0.19±0.05	0.26±0.06	0.14±0.05	0.11±0.04	p=0.68 (ns)
21/21	0.07±0.03	0.17±0.04	0.16±0.06	0.10±0.04	p=0.37 (ns)
21/22	0.15±0.05	0.26±0.07	0.16±0.06	0.16±0.05	p=0.54 (ns)
22/22	0.10±0.04	0.03±0.02	0.08±0.04	0.03±0.02	p=0.21 (ns)

between the hom(+) and hom(-) parents were in the pairwise satellite associations of 14/14 [p=0.02, hom(+)>hom(-)] and 15/15 [p=0.05, hom(+)>hom(-)].

Finally, satellite associations of the dNOR(+) homolog were assessed (Table 42). First, the dNOR(+) chromosomes of the parents of origin were compared to their normal homologs for the total number of satellite associations. Since there may have been different mechanisms by which homologous rearrangements and Robertsonian translocations arose, these groups were analyzed separately. In the dNOR(+) parents who contributed Robertsonian translocations, there were significantly more satellite associations of the normal chromosomes than their dNOR(+) homologs (X^2_1=11.0, $p<0.005$). However, in the parents who contributed homologous chromosome 21 rearrangements, there was no significant difference in the total number of satellite associations between the dNOR(+) chromosomes and their homologs (X^2_1=0.18, $0.75>p>0.50$).

Second, the satellite associations of the dNOR(+) chromosome were compared between those associations that involved the chromosomes in the resulting rearrangement and those of the other acrocentric chromosomes (Table 42). For example, in the parent of origin in family 2, there were three instances satellite associations which involved the dNOR(-) chromosome 15 and one which involved the dNOR(+) chromosome 15. The one association of the dNOR(+) homolog was with a chromosome 13; one of the possible chromosomes that resulted in the de novo rearrangement. The expected values for each

association was calculated based on the possible associations. For example, in family 2, the proband had a rob(13q14q) and the mother had a dNOR(+) 15. There were 4 of 45 possible associations of the dNOR(+) chromosome with the four chromosomes; 13A, 13B, 14A, and 14B. Since it was not known specifically which chromosomes, A or B, were involved in the rearrangement, both must be considered. Therefore, the chance of an association between the dNOR(+) chromosome 15 and chromosomes 13 or 14 was 4/45. Likewise, the chance that the dNOR(+) chromosome would associate with a non-chromosome 13 or 14 was 1-(4/45). In the parent who contributed a Robertsonian translocation, there were significantly more satellite associations of the dNOR(+) chromosomes with the chromosomes that gave rise to the de novo translocations than expected by chance ($X^2_5=28.14$, $p<0.005$). However, in the parents who contributed homologous chromosome 21 rearrangements, there was no significant difference between the satellite associations of the dNOR(+) chromosomes 21 with their homologs and the other acrocentric chromosomes ($X^2_3=6.41$, $0.10>p>0.05$) (Table 42).

Table 42. Comparison of the satellite associations of the dNOR(+) homolog

Robertsonian translocations

Family Number	dNOR(-) homolog	dNOR(+) homolog [observed(expected)]		
		other acro	rea chrom	Total
2	3	0 (0.91)	1 (0.09)	1
3	5	2 (1.87)	0 (0.13)	2
9	11	1 (1.82)	1 (0.18)	2
11	5	0 (0.93)	1 (0.07)	1
15	4	4 (3.64)	0 (0.36)	4
24	5	1 (0.91)	0 (0.09)	1
Total	33	8	3	11

Homologous rearrangements

Family Number	dNOR(-) homolog	dNOR(+) homolog [observed(expected)]		
		other acro	rea chrom	Total
1	8	9 (8.62)	5 (5.38)	14
5	4	5 (4.31)	2 (2.69)	7
6	3	0 (1.85)	3 (1.15)	3
13	8	2 (1.23)	0 (0.77)	2
Total	23	16	10	26

1. Satellite associations of dNOR(+) versus dNOR(-) homologs

rob $X^2_1 = 11.0$, $p < 0.005$

hom $X^2_1 = 0.18$, $0.75 > p > 0.50$

2. Satellite associations of dNOR(+) homolog with chromosomes of rearrangements versus other acrocentrics

rob $X^2_5 = 28.14$, $p < 0.005$

hom $X^2_3 = 6.41$, $0.10 > p > 0.05$

DISCUSSION

I. Distribution of the rearrangements

The distribution of the Robertsonian-type translocations is significantly different from random in our study with the majority accounted for by rob(13q14q), rob(14q21q) and rea(21q21q). This nonrandomness in chromosome participation in Robertsonian translocations has been documented in the literature for the last 20 years (Rowley and Pergament, 1969; Therman et al., 1989). Although nonrandom, the distribution found in our sample does not differ from the distribution of Therman et al. (1989). The apparent nonrandomness may result from an ascertainment bias or perhaps the formation of such translocations is not random.

An ascertainment bias exists in our sample since the majority of rearrangements were ascertained through Down syndrome parents organizations. Consequently, one would expect a large number of rearrangements involving chromosome 21. However, there is not a random distribution of all possible acrocentric rearrangements involving chromosome 21 since there are substantially fewer rob(13q21q), rob(15q21q) and rob(21q22q) than the numerous rob(14q21q) and rea(21q21q). It is possible that these under-represented Robertsonian

translocations do not result in Down syndrome as often as rob(14q21q) or rea(21q21q), which could explain their low ascertainment. However, these rearrangements are also under-represented in surveys of newborns and amniocenteses collected by Therman et al. (1989). Additionally, rea(21q21q) represents a large majority of the rearrangements seen in individuals with Down syndrome and is the major homologous acrocentric rearrangement ascertained. Again, this may reflect an ascertainment bias since rea(13q13q), rea(14q14q), rea(15q15q) and rea(22q22q) result in deleterious phenotypes that are rarely seen in newborns. However, these rearrangements are rarely seen in surveys of abortuses (Hassold, 1980) which supports the idea that these rearrangements rarely form. Therefore, the large number of rea(21q21q) that are ascertained from surveys of individuals with Down syndrome may reflect a real increase in formation of these rearrangements. Since rea(21q21q) are the most frequently occurring *de novo* rearrangements in Down syndrome, these rearrangements may form through more than one mechanism. Some may form through mechanisms that give rise to "true" Robertsonian translocations while others may form through mechanisms that give rise to isochromosomes.

Finally, individuals who carry rob(13q14q) are over-represented in our study. The majority of individuals who carry balanced Robertsonian translocations were ascertained through the cytogenetic records in the Department of Human Genetics at the Medical College of Virginia. Most of these

individuals were referred for cytogenetic study because of multiple miscarriages or an abnormal amniocentesis. The distribution of the rearrangements in the individuals who decided to participate in the study reflect the distribution of the rearrangements in the cytogenetic records as a whole and may reflect the population of translocation carriers in the Richmond, Virginia area. Since individuals were ascertained solely on the assumption that they carry a Robertsonian translocation and not on the chromosomes involved in the rearrangement, the balanced carriers ascertained most probably reflect the true distribution of these rearrangements in the population since it is reasonable to assume that carriers of rob(13q14q) are no more likely to participate in scientific investigations than carriers of other Robertsonian translocations. Although the observations of the chromosomal distribution in Robertsonian translocations in our study do not explain the nonrandomness observed, our study does reflect the distributions previously published and seems representative of most individuals who carry Robertsonian-type rearrangements.

II. Parental origin of de novo acrocentric rearrangements

Cytogenetic heteromorphisms have been useful tools for the study of parental origins of human trisomies. We have employed these variants to assign the parental origins of de novo acrocentric rearrangements. The combined use of the QFQ, NOR and morphological cytogenetic heteromorphisms was demonstrated to be very effective in assigning the parental

origins in 84% of families studied. For the "true" Robertsonian translocations, 92% of the parental origins could be assigned. Fewer of the origins could be assigned in the homologous rearrangements (75%). This may be expected since the parental origin assignments of these rearrangements are based on a single free-lying chromosome. However, in this study, we were able to assign the origins in almost twice as many rea(21q21q) than a previous report in which only 40% could be assigned using QFQ and NOR heteromorphisms (Nikolis and Kekic, 1986).

Restriction fragment length polymorphisms (RFLPs) have been used by other investigators to assign the parental origin and meiotic stage of the nondisjunctional error resulting in trisomy 21. We have applied these techniques to assign the parental origins of de novo acrocentric rearrangements resulting in trisomy. The use of RFLPs proved to be very valuable for assigning parental origins. The origins could be assigned in 86% of families studied. Of the Robertsonian translocations tested, 60% of the origins could be assigned. All of the parental origins of the homologous rearrangements could be assigned in addition to the one case of trisomy 21.

The use of RFLP analysis in this study demonstrates the utility of this tool. Additionally, the results of the RFLP analyses confirm the accuracy and usefulness of the cytogenetic markers for determining parental origins of acrocentric rearrangements since the assignments agreed in every case in which both methods were studied. Therefore,

using the combined cytogenetic and molecular markers, the parental origins of all _de novo_ rearrangements could be assigned. There was an equal distribution between maternally and paternally derived rearrangements for the combined sample. Although there was no significant difference in the parental origin distribution of the "true" Robertsonian translocations, there were more maternally derived rearrangements (62%) which agreed with nine previously reported cases (Table 7). Additionally, albeit not statistically significant, there was a 2-fold increase in the number of paternally derived homologous rearrangements in this study. In twenty-six previously reported cases of rea(21q21q) (Table 8), there were slightly more maternally derived cases of rea(21q21q). However, there was no difference in the distribution of all homologous rearrangements previously reported (Table 8). In our case of a "mirror image" chromosome, the rearrangement was found to be paternally derived. Two other "mirror image" rearrangements of chromosome 21 were also paternally derived (Table 8).

The number of parental origin assignments of _de novo_ acrocentric rearrangements determined previous to this study are small. However, in general, these previous findings agree with the distribution of the parental origins in this study. The results in the present study suggest that more Robertsonian translocations are maternally derived. The mechanisms by which the _de novo_ Robertsonian translocations form may be the same as those which have been postulated to

cause maternal meiosis I nondisjunction. In contrast, the mechanisms which give rise to homologous rearrangements or isochromosomes may occur more frequently in males. Although either of these rearrangements can occur in males or females, male and female gametogenesis occur differently. By examining meiosis in each, one may understand how the process of meiosis could give rise to a preponderance of maternally derived Robertsonian translocations and an increased incidence of paternally derived homologous rearrangements.

Meiosis in the human oocyte begins early in fetal development. At leptotene, each oocyte has several nucleoli, each corresponding to an active nucleolar organizer. By pachytene, the nucleolar organizers have fused forming about two nucleoli per nucleus. The majority of the nucleoli are associated with one bivalent. However, in approximately 40% of oocytes, nucleoli are associated with two or three nonhomologous bivalents (Mirre et al., 1980). The nucleoli persist during diplotene. Therefore, the oocytes maintain the acrocentric associations in the nucleoli until ovulation, 12-40+ years later. During these associations, breakage and reunion of nonhomologous chromosomes could lead to the formation of Robertsonian translocations. In contrast, during meiosis in the human spermatocyte, only 20% of spermatocytes have two or more bivalents associated in the same nucleolus (Stahl et al., 1983). Therefore, there would appear to be much less of an opportunity for Robertsonian translocations to occur between nonhomologous chromosomes in spermatogenesis

than in human oogenesis. An additional difference in male and female gametogenesis is that spermatocytes are produced continuously after puberty in the male and there is no interruption during prophase I in spermatogenesis as in oogenesis. This may further reduce the opportunity for Robertsonian translocations to form in the male.

It may be fortuitous that males were found to have been the parent of origin for the majority of homologous rearrangements of chromosome 21. However, it is known that the recombination rates for chromosome 21 vary along the length of the chromosome and between the sexes (Tanzi et al., 1988; Warren et al., 1989) and this may contribute to the sex differences in the risk of forming homologous rearrangements of chromosome 21, specifically isochromosomes. Females have approximately twice the recombination of males in the proximal region of the long arm (near the centromere) while both have similar recombination rates in the distal region of 21q (Tanzi et al., 1988; Warren et al., 1989). It is possible that the reduced amount of recombination between the bivalents allows for increased amounts of sister chromatid exchanges (intra-recombination) in the pericentromeric region of chromosomes 21 in males. The increase of exchanges between sister chromatids may facilitate erroneous U-type reunions resulting in isochromosomes.

The assignments of parental origin were based on two assumptions. First, the _de novo_ rearrangement occurred as a result of a meiotic error and not a post-zygotic mitotic

event. Thus, the de novo rearrangement was inherited from one parent and the corresponding free-lying chromosomes were inherited from the other parent. Second, the cytogenetic heteromorphisms are stably inherited and crossing over in the pericentromeric region is negligible.

Regarding the first assumption, if the translocations formed post-zygotically in individuals who have balanced de novo translocations, mosaicism may be expected. Although only one tissue type was examined, no evidence of mosaicism was present in the 10 cells examined in each of five probands who had balanced Robertsonian translocations (probands 2, 7, 9, 11 and 25). By examining 10 cells, mosaicism of 26% or greater can be excluded at the 0.95 confidence level (Hsu, 1986). Additionally, no cases of mosaicism have been reported for carriers of de novo "balanced" Robertsonian translocations. Furthermore, if the event had occurred post-zygotically, there would have been a random chance of any chromosomes becoming translocated including the chromosomes inherited from different parents. In our sample, the free-lying homologous chromosomes to the de novo balanced Robertsonian translocation came from the same parent in every case (Table 20).

In individuals who had unbalanced Robertsonian translocations leading to tertiary trisomy, a random post-zygotic event could lead to two free-lying homologous chromosomes from one parent. In the majority of cases examined in this study, the two free-lying chromosomes could

be identified as having been inherited one from each parent. However, in families 12, 23 and 24, this could not be determined. Additionally, in family 12, the molecular analyses were inconclusive. The majority of chromosome 21 loci tested in this family were either uninformative or indeterminate and one locus, D21S15, was consistent with non-maternity (Fig. 11). One explanation for these findings is that the child is not the alleged mother's child. This possibility was not explored with the parents and cannot be ruled out. "Paternity" testing with non-21 RFLPs were consistent within this family. Additionally, some difficulty was experienced with this sample in the hybridizations with several other probes and the results of some autoradiographs were unclear (i.e. D21S112, see Fig. 10). Thus, it is not known at this time if these results truly reflect the molecular constitution of the proband or if they can be attributed to artifact.

In regards to the second assumption for assigning parental origins, the short arms of the acrocentric chromosomes are thought to rarely undergo recombination based on a study of chiasmata frequency of over 800 acrocentric chromosomes (Laurie and Hultén, 1985). The D group chromosomes had evidence of short arm chiasmata in less than 2% of chromosomes examined. The G group chromosomes had evidence of short arm chiasmata in less than 0.5% of chromosomes studied. Therefore, it is reasonable to assume that crossing over in the acrocentric short arms is

negligible. However, to eliminate any biases which may occur because of undetected crossovers, subjectivity from the investigator or unreliable staining, RFLP analysis was used to supplement the cytogenetic markers. In this investigation, seven families were studied in which both cytogenetic and RFLP analyses were conclusive. The analyses were performed blinded to one another and in each case studied, the origin assignments agreed (Table 23). Therefore, the cytogenetic markers could be used to accurately and reliably determine the parental origins of the de novo acrocentric rearrangements.

III. Factors which may influence Robertsonian translocation and Isochromosome formation

In this study, a dNOR variant was noted in a significantly higher proportion of parents who had a child with a de novo acrocentric rearrangement as compared to the control group. A dNOR variant chromosome has only been reported once previously in a mother who had two children with de novo rea(21q21q) (Jackson-Cook et al.,1988). Additionally, two cases of elongated acrocentric short arms have been associated with de novo Robertsonian translocations (Table 9) (Jacobs et al., 1974; Pérez-Castillo and Abrisquetta, 1978).

If the dNOR variant is involved with the formation of de novo acrocentric rearrangements, one would expect it to be found in the parent in whom the rearrangement originated. In our study, when the dNOR variant was present, it was invariably found in the parent of origin. The dNOR variant was found both in parents who contributed "true" Robertsonian

translocations as well as those in whom *de novo* homologous rearrangements originated.

Possible roles of the dNOR variant in acrocentric rearrangement formation include 1) enhancing nucleolar persistence to form Robertsonian translocations; 2) causing recombination between nonhomologous chromosomes to form Robertsonian translocations; 3) promoting U-type reunions between sister chromatids to form isochromosomes; and 4) enhancing nucleolar persistence which leads to univalents that undergo intra-chromosomal exchanges to form isochromosomes.

Ohno et al. (1961) was the first to hypothesize that during nucleolar persistence, breakage and exchange could occur between the acrocentric chromosomes. Miller et al. (1978) found that in mice, the chromosomes with the active NORs were more likely to be involved in Robertsonian translocations than those chromosomes with inactive or absent NORs. In this study, we were able to test this indirectly by examining if the more active NORs (dNORs) were involved in Robertsonian translocations more often than the other chromosomes. Although there was no significant difference between the parents of origin and their normal spouses for overall 1) mean NOR score or 2) mean number of silver-positive chromosomes, the parents of origin had significantly higher mean NOR scores for chromosome 21 than their spouses or controls. Approximately 50% of the dNOR(+) variants in the parents of origin were on chromosomes 21 which accounts for the finding that a NOR score of 4 or greater was found most

frequently on chromosome 21 in the dNOR(+) parents. Additionally, chromosome 21 was involved in 76% of the de novo rearrangements. However, there was no significant difference between the dNOR(+) and dNOR(-) parents of origin with respect to total mean NOR score, mean number of silver-positive chromosomes or mean NOR score for any specific acrocentric chromosome.

Nucleolar persistence is thought to be visualized in mitotic cells as satellite associations. The rationale is that if satellite associations are an individual yet stable trait (Phillips, 1975; Yip and Fox, 1981), then a correlation can be drawn between what is viewed in the mitotic cells and what could be happening in the meiotic cells. Acrocentric chromosomes with long stalk lengths have been found to participate in satellite associations more frequently than their homologs (Schmid et al., 1974; Miller et al., 1977; de Capoa et al., 1978; Di Lernia et al., 1980). Additionally, dNOR variants have been shown by in situ hybridization with labelled rRNA to have up to six times the silver grains as other acrocentric chromosomes suggesting that they contain more copies of rRNA genes than the other chromosomes (Miller et al., 1978). One might assume that the greater the number of active rRNA genes, the greater the tendency to enter into satellite associations. However, several studies have not seen an increased satellite association with NOR variants (Evans et al., 1974; Miller et al., 1978; Bernstein et al., 1981).

In this study, satellite associations were examined to investigate if the dNOR variant was enhancing satellite associations that could lead to the formation of Robertsonian translocations. There were no significant differences between the parent of origin, their normal spouses and controls for 1) mean number of satellite associations; 2) mean number of chromosomes that participated in satellite associations; 3) mean number of chromosome per satellite association; 4) mean association index; or 5) mean pairwise satellite association except for 21/22 SA were greater in the controls and 22/22 SA were greater in the spouses. Furthermore, although the parents of origin had higher mean NOR scores for chromosome 21, they did not have a higher mean association index for chromosome 21. The additional findings that there were no significant differences in the dNOR(+) or dNOR(-) parents of origin for 1) mean satellite associations, 2) mean number of chromosomes in satellite associations, 3) mean number of chromosomes per satellite associations, 4) mean AI, or 5) mean pairwise associations support the observations of the previous investigations that the dNOR does not enhance satellite associations (Evans et al., 1974; Miller et al., 1978; Bernstein et al., 1981). Additionally, when the dNOR(+) chromosomes were compared to their normal homologs, the normal homologs entered into significantly more satellite associations than the dNOR(+) chromosomes also supporting the findings of other investigators (Evans et al., 1974; Miller et al., 1978; Bernstein et al., 1981). However, when the

specific chromosomes involved in the satellite association with the dNOR(+) homologs were examined, there were significantly more associations with the chromosomes that gave rise to the de novo Robertsonian translocation than expected by chance. In summary, our results do not support an increase in satellite associations due to the presence of dNOR variants in the parents of origin group. However, the dNOR(+) chromosome may participate in specific satellite associations that could lead to the formation of Robertsonian translocations. During these specific satellite associations, the dNOR(+) variant may facilitate exchanges between non-homologous chromosomes that come into close association because of shared homologous regions. Thus, the second possible role of the dNOR variant is that it may facilitate recombination between nonhomologous chromosomes to form Robertsonian translocations.

The acrocentric chromosomes share several classes of DNA in common in their pericentromeric and stalk regions: rRNA genes, the 724 gene family (Kurnit et al., 1986), alpha satellite DNA and satellite DNA I-IV (Choo et al., 1989). Many of these DNA sequences have been shown to hybridize to all five human acrocentric chromosomes (Kurnit et al., 1984; Willard, 1985; Devine et al., 1985; Kurnit et al., 1986). These DNA classes have been postulated to have been dispersed over the acrocentric chromosomes through unequal sister chromatid exchanges between nonhomologous chromosomes (Kurnit, 1979). Choo et al. (1989) have speculated on a model for

Robertsonian translocation formation. The model is based on the assumption that exchanges can occur between nonhomologous chromosomes. DNA sequences have been identified that are common to all and unique to some acrocentric chromosomes. The homology and repetitive nature of these sequences may facilitate the homologous pairing of nonhomologous chromosomes. We can further speculate that the enriched rRNA genes on the dNOR variant chromosome may facilitate nonhomologous exchanges. Some of these exchanges may lead to Robertsonian translocation formation.

The model presented by Choo et al. (1989) is attractive since it also accounts for the nonrandom participation of the acrocentric chromosomes in Robertsonian translocations. It has been postulated that the pericentromeric sequences shared between chromosomes 13, 14 and 21 are inverted on chromosome 14 compared to the orientation of these sequences on chromosomes 13 and 21 (Therman, 1980; Choo et al., 1988). This would explain the appearance of rob(13q14q) and rob(14q21q) but the absence of rob(13q21q). We can further speculate that the dNOR variant is an inverted repeated segment of tandemly repeated units of rRNA genes. The NOR is composed of tandemly repeated genes for the 18s and 28s ribosomal RNA. The dNOR variant appears morphologically as a doubling or duplication of the NOR as visualized by an ammoniacal silver stain with an elongated stalk region when viewed with QFQ (Jackson-Cook et al., 1985) (Fig. 7). The dNOR variant has not been characterized with molecular

techniques. It is possible that the dNOR is an inverted segment of tandemly repeated rRNA genes and the inverted orientation would further facilitate nonhomologous exchanges through increased homologous pairing of nonhomologs. Although we cannot offer any data at this time to support this speculation, this model is nevertheless attractive and worthy of further investigation.

In order to further understand the interactions between the acrocentric chromosomes, one may want to examine recombination to assess if a de novo Robertsonian translocation can pair properly with its homologs. A recombinational event may indicate that proper pairing was achieved. The recombination in the parent who contributed the de novo Robertsonian translocation can be compared with the homologs inherited from the non-contributory parent in the proband and the siblings. In our study, recombination could be detected between the chromosomes inherited from the parent of origin in four probands who had Robertsonian translocations (Fig. 10). In two, a recombinational event occurred between the "centromeric" marker D21S13 and the cytogenetic markers (families 15 and 16). This interpretation assumes that the chromosomes involved in the translocations were not the same as those inherited as free-lying chromosomes. Consequently, since recombination was evident, proper pairing was achieved in at least four families studied. Additionally, recombination could be detected in several of the siblings in two of these families. However, none of these families were

large enough to make definitive statements about the number of recombinations per chromosome inherited since it was virtually impossible to identify the particular recombinant chromosomes from nonrecombinants among the siblings. We could only state that a recombinational event had occurred

We have used a narrow and strict definition of an isochromosome as a chromosome composed of genetically identical arms, derived from one parental chromosome (Darlington, 1939; 1940). Homologous rearrangements of chromosome 21 have most commonly been referred to as rob(21q21q). The distinction between isochromosomes and Robertsonian translocations could not be established until the availability of RFLP analyses. In this study, we have employed the techniques of RFLP analysis, used previously on only trisomy 21, to distinguish between Robertsonian translocations and isochromosomes. The results of the RFLP analyses suggest that all rea(21q21q) were isochromosomes. Additionally, the "mirror image" rearrangement in proband 26 was also consistent with an isochromosome. Thus, our results indicate that the majority of homologous exchanges of chromosome 21 are isochromosomes.

Several mechanisms of isochromosome formation have been postulated. The two most commonly suggested mechanisms are misdivision of the centromere (Darlington, 1939; 1940) and a U-type reunion between sister chromatids (de la Chapelle et al., 1966). While misdivision of the centromere would result in only monocentric products, U-type reunions between sister

chromatids could result in dicentric or monocentric isochromosomes. A monocentric isochromosome could occur if the exchange took place within the chromatin of the centromere and dicentric isochromosomes could result from exchanges in the short arm or NOR (Van Dyke, 1988). Since four of the probable isochromosomes were dicentric, our results suggest that U-type exchanges between sister chromatids may be the predominant, if not exclusive, mechanism by which both monocentric and dicentric isochromosomes form.

With respect to the third role of the dNOR variant in specifically isochromosome formation, as proposed by Holden et al. (1989), inverted repeated segments in the centromere, short arm or the NOR may facilitate sister chromatid exchanges resulting in isochromosomes. In our sample, four parents of origin had dNOR(+) chromosomes 21 and a child with rea(21q21q). Only one family was studied by RFLP analysis. In this family, the dNOR(+) chromosome 21 was shown to be the chromosome that was inherited as an idic(21q) in the child (Fig. 12). As discussed previously, it is possible that at least some dNOR variants are inverted duplications of tandemly repeated units. Inverted repeats of tandemly repeated segments, such as homogeneously staining regions (HSRs), have been shown to give rise to isochromosomes through unequal exchanges between sister chromatids (Holden et al., 1989).

Finally, the fourth possible mechanism by which the dNOR variant may contribute to isochromosome formation is by nucleolar persistence. Nucleolar persistence could inhibit

the dNOR(+) chromosome from pairing with its homolog. In the absence of proper pairing, either univalent could undergo an intra-chromosomal exchange that leads to the formation of an isochromosome. Univalents in the meiotic cells of plants have been observed to form isochromosomes (Darlington, 1939). Support for this role of the dNOR variant comes from our observation of dNOR(+) chromosomes 21 in all dNOR(+) parents who contributed a homologous chromosome 21 rearrangement. As mentioned previously, using RFLP analysis in family 1, the dNOR(+) chromosome 21 was shown to be the chromosome that formed the isochromosome. However, under this hypothesis, either univalent may form an isochromosome.

In summary, the results of this study indicate that the majority of homologous rearrangements of chromosome 21 are isochromosomes. This finding is in agreement with Grasso et al. (1989) who recently found that four of six rea(21q21q) are maternally derived isochromosomes. Unlike their conclusions however, results of the C-banding in this study suggest that an exchange between sister chromatids with a U-type reunion occurs at least as often as centromere misdivision and could possibly occur more frequently resulting in both monocentric and dicentric isochromosomes. Grasso et al. (1989) assumed that the finding of recombination in two of the probable isochromosomes must have resulted from misdivision of the centromere prior to meiosis. However, as explained subsequently, detecting recombination does not inevitably lead to the conclusion of misdivision of the centromere.

The evaluation of recombination in families who have children with rea(21q21q) can possibly help one understand when these rearrangements occurred in the cell cycle and may help to distinguish between isochromosomes and Robertsonian exchanges. Homologous rearrangements can occur as "true" Robertsonian translocations between homologous chromosomes or as isochromosomes (an intra-chromosomal rearrangement). If a Robertsonian translocation occurred prior to bivalent formation, recombination may not occur because of physical constraints of the newly formed rearrangement. Furthermore, if an isochromosome 21 forms during meiotic pairing, it is not known if this isochromosome can undergo further recombination. "Isochromosomes" have been observed to fold back on themselves to permit pairing of homologous segments of DNA in the meiotic cells of maize (Rhoads, 1940) and tomato (Sen, 1952). This "internal pairing" was seen more frequently than other pairing configurations in tomato (Sen, 1952).

Since no rob(21q21q) were ascertained, we were unable to evaluate recombination in these rearrangements. However, in this study, recombination was detected in one isochromosome. On the average, chromosome 21 has a mean chiasma frequency of 1.06 (Laurie and Hultén, 1985). Of the 199 chromosomes examined, approximately 93% had one chiasmata, 5.5% had two chiasmata and 1% had no detectable chiasmata (Laurie and Hultén, 1985). If we assume that isochromosomes form predominantly through a U-type reunion between sister chromatids, one crossover has occurred in the short arm or

centromere in forming the isochromosome. Since only 5.5% of bivalents would be expected to have two chiasmata, our observation of one rearrangement in eight (12.5%) in which an additional crossover occurred, is not significantly different from the expected based on the experimental data by Laurie and Hultén (1985) (X^2_1=0.75, 0.50>p>0.25). However, there is no evidence to suggest that a recombinational event in the short arm could indeed interfere with recombination in the long arm on chromosome 21. Furthermore, the deficiency of recombination detected in distal 21q of the isochromosomes, may likely be due to the uninformative or inconclusive results in these families for many of the DNA markers tested. However, recombination was detected in the free-lying chromosomes 21 in three families tested. Although recombination should have occurred in 93% of the chromosomes studied, it is possible that it could not be detected given the lack of markers used in distal 21q.

Since the majority of rea(21q21q) were determined by RFLP analysis to be isochromosomes (intra-chromosomal rearrangements), satellite associations (an inter-chromosomal event) are not likely to play a significant role in their formation. Our results of no significant differences between the parents of origin, their spouses or controls for mean 21/21 satellite associations support the hypothesis that satellite associations do not play a role in rea(21q21q) formation. Additionally, there were no significant differences between the 21/21 pairwise satellite associations

between the rob and hom parents. Satellite associations of homologous chromosomes 21 were also found to be not significant in a study of 10 parents who had children with de novo rea(21q21q) (Nikolis and Kekic, 1986). However, in the present study, there were significantly more 13/13 satellite associations in the parents of origin who contributed homologous rearrangements than in the parents who contributed Robertsonian translocations. Additionally, the dNOR(+) parents who contributed homologous rearrangements had significantly more 14/14 and 15/15 satellite associations than the hom(-) parents. These findings suggest that there may be different mechanisms that give rise to homologous rearrangements of the D group chromosomes compared to rea(21q21q). Possibly, the majority of rea(21q21q) are i(21q) while homologous rea(DqDq) are truly Robertsonian translocations.

IV. Compensation

The nonrandom distribution of the acrocentric chromosomes in Robertsonian-type translocations may be understood by examining compensation. Compensation refers to a mechanism by which other acrocentric chromosomes not involved in the rearrangement increase their activity to make up for the absent NORs lost in the formation of the rearrangement. In general, there has been little evidence offered in favor of a compensatory mechanism (Zankl and Hahmann, 1978; Jotterand-Bellomo and Van Melle, 1981; Nikolis et al., 1981). However, compensation was documented by the assessment of NOR staining

in a proband with a <u>de novo</u> t(13q14q) (Gosden et al., 1979) and in a study of probands with rea(21q21q) (Nikolis and Kekic, 1988). In the present study, compensation was evaluated by comparing the mean NOR scores and mean number of silver-positive chromosomes between the parents and the offspring who had <u>de novo</u> Robertsonian-type rearrangements. Although the parents had significantly higher mean NOR scores and mean number of silver-positive chromosomes than the probands, there was no significant difference in the mean NOR score per silver-positive chromosome between the parents and offspring (Table 30). Therefore, our results do not support a compensatory mechanism in these probands. However, these conclusions are based on the assumption that the total NOR score, by silver staining, correlates positively with the incorporation of ^{3}H-uridine into nucleolar rRNA as demonstrated by Morton et al. (1983). Additionally, the parents had significantly higher mean NOR scores than the control individuals. This finding may not be relevant given the sample size of our study; however, since all but one of these families were ascertained through a child with a <u>de novo</u> rearrangement of the acrocentric chromosomes and the probands may have lost active NORs, our findings of increased NOR scores in the parents may reflect a selection process which is attributable to the fetus' inheritance of active NORs on their acrocentric chromosomes not involved in the rearrangement. Thus, the fetuses from these parents possibly have a greater ability to survive than fetuses with <u>de novo</u>

acrocentric rearrangements from parents with lower NOR scores.

Additional evaluations for a compensatory mechanism in probands with de novo acrocentric rearrangements comes from the study of satellite associations. In order for a de novo rearrangement to result in viable offspring, it may be important to maintain an undefined threshold level of satellite associations. A study of 12 individuals of varying Robertsonian-type translocations demonstrated that the free-lying homologous chromosomes of the rearrangement participated in satellite associations more often than the other acrocentric chromosomes (Hansson, 1975). In our study, when the parents and offspring were compared, there were no significant differences in mean number of satellite associations and mean number of chromosomes per satellite association. However, as expected, the probands had significantly fewer chromosomes available for satellite associations and thus had a lower mean number of chromosomes that participated in satellite associations. However, when the mean association indexes (AI) were compared (the AI takes into account the number of chromosomes per cell), there were no significant differences noted for any acrocentric AI between the parents and offspring. Additionally, the probands never had a higher pairwise satellite association than the parents and had several that were significantly lower than the parents. Therefore, our results do not suggest a compensatory mechanism for satellite associations in the probands.

V. Recurrence risks

The recurrence risks of <u>de novo</u> acrocentric rearrangements may result from 1) a true recurrence of the <u>de novo</u> event due to a genetic predisposition; 2) recurrence by chance alone; or 3) parental mosaicism. Although gonadal mosaicism can rarely be excluded, the risk of having a second child with <u>de novo</u> translocation Down syndrome ranges from 1% (Gardner and Veale, 1974) to 2% (Steinberg et al., 1984). There have been reports of rea(21q21q) recurrences to apparently non-mosaic, chromosomally normal individuals (Garver et al., 1982; Schmidt and Nitowsky, 1977; Jackson-Cook et al., 1988). The recurrence risks for specifically i(21q) and rob(21q21q) <u>de novo</u> rearrangements have not been examined most likely because of their rare occurrence. In this study, none of the parents had a second child with an acrocentric rearrangement although family 8 had a conception with a 6p+ and family 18 had a conception with trisomy 21. Based on the observation of one in 21 subsequent pregnancies resulting in an acrocentric chromosome abnormality, the poisson confidence limits were obtained using the methods of Burstein (1971). In this study, the risk of any acrocentric chromosome abnormality in a subsequent pregnancy ranged from 0.1% to 24%. The dNOR variant has been implicated as a causal factor in nondisjunction associated with Down syndrome (Jackson-Cook et al., 1985; Melnyk et al., 1987). This variant may be associated with a 6-8 fold increase risk for having a child with Down syndrome (Jackson-Cook, 1990).

However, other studies indicate that the dNOR variant does not contribute to nondisjunction of chromosome 21 (Hassold et al., 1987; Spinner et al., 1989). The differences in these studies may result from the definition and identification of the variants in addition to disparity in silver staining techniques between the investigators. In the present study, the dNOR variant appeared to be associated with a 7-fold increase for having a child with a de novo acrocentric rearrangement. However, the dNOR variant does not appear to be an efficient means of screening couples who may be at risk for having a child with a de novo acrocentric rearrangement since the absolute risk, estimated to be 1 in 5000 newborns, is low.

In conclusion, the dNOR variant was found to be a possible factor in causing de novo acrocentric rearrangements in approximately 40% of families studied. Since the dNOR variant was found both in families who have a child with a de novo Robertsonian translocation and those who have a child with an isochromosome, further evaluation of the dNOR variant could provide valuable information for genetic counseling for these families and their relatives.

Since the majority of families were dNOR(-), other unknown factors may be placing subsequent pregnancies at risk. The empiric recurrence risks ranged from 1-3.6% (Gardner and Veale, 1974; Steinberg et al., 1984). Since these risks are higher than the risks associated with prenatal testing (~1/350 for amniocentesis at MCV), all families should be offered

prenatal testing.

Finally, and most unexpectedly, our results indicate that the majority of homologous rearrangements may be isochromosomes. Further study of carriers of rearrangements for chromosome 21 may provide important clues to phenotypic effects of isochromosome Down syndrome, translocation Down syndrome and trisomy 21. Looking ahead to future studies, isochromosomes provide a unique opportunity to study the effects of homozygosity on a phenotype and the potential risks for diseases such as leukemia.

CONCLUSIONS

The primary conclusions from this study were:

1) The participation of the acrocentric chromosomes in Robertsonian type rearrangements in this study were nonrandom but did not differ from previous surveys of Robertsonian-type rearrangements.

2) By using both molecular and cytogenetic techniques, the parental origins of all de novo rearrangements could be assigned (26/26).

3) No significant difference was noted in the proportion of de novo acrocentric rearrangements from males versus females (12 mat: 13 pat). There was a trend towards more maternally derived Robertsonian translocations (8 mat: 5 pat) and more paternally derived homologous rearrangements (8 pat: 4 mat).

4) The majority of homologous rearrangements of chromosome 21 may be isochromosomes based on our finding that all probands studied with RFLP analysis were found to carry i(21q).

5) The major mechanism by which isochromosomes form was concluded to be a U-type reunion between sister chromatids based on the observation of both monocentric and dicentric isochromosomes by C-banding.

6) Recombination was detected in 4/6 of the probands who had de novo Robertsonian translocations, suggesting that proper pairing was achieved between the de novo rearrangement and its free-lying homologs in the parents who contributed Robertsonian translocations. Additionally, recombination was detected in 1/8 isochromosomes suggesting that recombination can occur in addition to the formation of the isochromosome.

7) The dNOR variant was identified as a possible factor in the formation of acrocentric rearrangements in 11/25 families. Additionally, the dNOR chromosome was invariably in the parent in whom the de novo acrocentric rearrangement originated. The dNOR variant was found both in parents who contributed de novo Robertsonian translocations and homologous rearrangements. Thus, suggesting that the dNOR variant may have played a role in the formation of both types of acrocentric rearrangements.

8) Overall satellite associations did not appear to play a role in Robertsonian translocation formation. However, the dNOR(+) chromosome may participate in specific satellite associations that could lead to the formation of Robertsonian translocations.

9) The relative risk of having a child with a de novo acrocentric rearrangement appeared to be 7 times higher among the dNOR(+) individuals as compared to the dNOR(-) individuals.

REFERENCES

REFERENCES

1. Abe, T, Morita, M, Kawai, K, Misawa, S, Kanai, H, Hirose, G, and Fujita, H (1975) Transmission of a t(13q22q) chromosome observed in three generations with segregation of the translocation D1-trisomy syndrome. Humangenetik 30:207-215.

2. Abeliovich, D, Katz, M, Karplus, M, and Carmi, R (1985) A de novo translocation, 14q21q, with a microchromosome-14p21p. Am J Med Genet 22:29-33.

3. Arnheim, N, Krystal, M, Schmickel, R, Wilson, G, Ryder, O, and Zimmer, E (1980) Molecular evidence for genetic exchanges among ribosomal genes on nonhomologous chromosomes in man and apes. Proc Natl Acad Sci 77:7323-7327.

4. Atkins, L, and Bartsocas, CS (1974) Down's syndrome associated with two Robertsonian translocations 45,XX,-15,-21,t(15q21q) and 46,XX,-21,t(21q21q). J Med Genet 11:306-309.

5. Balicek, P, Zizka, J, and Skalska, H (1982) RHG-band polymorphism of the short arms of human acrocentric chromosomes and relationship of variants to satellite associations. Hum Genet 62:237-239.

6. Balkan, W, and Martin, RH (1983) Segregation of chromosomes into the spermatozoa of a man heterozygous for a 14;21 Robertsonian translocation. Am J Med Genet 16:169-172.

7. Becher, R, Mahmoud, HK, Schaefer, UW, and Schmidt, CG (1985) Ph-positive CML in a family with a constitutional Robertsonian translocation 14;15. Cancer Genet Cytogenet 18:229-234.

8. Becher, R, Wendt, F, and Kuhn, D (1987) Constitutional Robertsonian T(15;22) in Ph-positive CML. Hum Genet 76:399.

9. Benn, PA, and Perle, MA (1986) Chromosome staining and banding techniques. In D.E. Rooney and B.H. Czepulkowski(eds): Human Cytogenetics: A Practical Approach. Washington,D.C.: IRL Press, pp. 61-71.

10. Berland, HM, Sharma, A, Cribiu, EP, Darre, R, Boscher,J, and Popescu, CP (1988) A new case of Robertsonian translocation in cattle. J Hered 79:33-36.

11. Bernstein, R, Dawson, B, and Griffiths, J (1981) Human inherited marker chromosome 22 short-arm enlargement: Investigation of rDNA gene multiplicity, Ag-band size, and acrocentric association. Hum Genet 58:135-139.

12. Birnboim, HC, and Doly, J (1979) A rapid alkaline extraction procedure for screening recombinant plasmid DNA. Nucleic Acids Res 7:1513-1523.

13. Bloom, SE, and Goodpasture, C (1976) An improved technique for selective silver staining of nucleolar organizer regions in human chromosomes. Hum Genet 34:199-206.

14. Bonaminio, GA, and Fechheimer, NS (1988) Segregation and transmission of chromosomes from a reciprocal translocation in Gallus Domesticus cockerels. Cytogenet Cell Genet 48:193-197.

15. Boué, J, and Boué, A (1973) Chromosomal analysis of two consecutive abortuses in each of 43 women. Humangenetik 19:275-280.

16. Bruere, AN (1975) Further evidence of normal fertility and the formation of balanced gametes in sheep with one or more different Robertsonian translocations. J Reprod Fert 45:323-331.

17. Bruere, AN, Scott, IS, and Henderson, LM (1981) Aneuploid spermatocyte frequency in domestic sheep heterozygous for three Robertsonian translocations. J Reprod Fert 63:61-66.

18. Burstein, H. (1971) Attribute Sampling: Tables and Explanations. McGraw-Hill Book Company, New York, 1971.

19. Campana, M, Serra, A, and Neri, G (1986) Role of chromosome aberrations in recurrent abortion: A study of 269 balanced translocations. Am J Med Genet 24:341-356.

20. Capanna, E, Cristaldi, M, Perticone, P, and Rizzoni, M (1975) Identification of chromosomes involved in the 9 Robertsonian fusions of the Apennine mouse with a 22-chromosome karyotype. Experientia (Basel) 31:294-296.

21. Capanna, E, Gropp, A, Winking, H, Noack, G, and Civitelli, MV (1976) Robertsonian metacentrics in the mouse. Chromosoma 58:341-353.

22. Carter, CO, Hamerton, JL, Polani, PE, Gunalp, A, and Weller, SDV (1960) Chromosome translocation as a cause of familial mongolism. Lancet 2:678-680.

23. Caspersson, T, Hulten, M, Lindsten, J, Therkelsen, AJ, and Zech, L (1971) Identification of different Robertsonian translocations in man by quinacrine mustard fluorescence analysis. Hereditas 67:213-220.

24. Caspersson, T, and Zech, L (1971) The 24 fluorescence patterns of the human metaphase chromosomes-distinguishing characters and variability. Hereditas 67:89-102.

25. Cattanach, BM (1986) Parental origin effects in mice. J Embryol Exp Morph 97:137-150.

26. Chakravarti, A (1989) The probability of detecting the origin of nondisjunction of autosomal trisomies. Am J Hum Genet 44:639-645.

27. Chamberlin, J, and Magenis, RE (1980) Parental origin of de novo chromosome rearrangements. Hum Genet 53:343-347.

28. Chapman, HM, and Bruere, AN (1975) The frequency of aneuploidy in the secondary spermatocytes of normal and Robertsonian translocation-carrying rams. J Reprod Fert 45:333-342.

29. Choo, KH, Vissel, B, Brown, R, Filby, RG, and Earle, E (1988) Homologous alpha satellite sequences on human acrocentric chromosomes with selectivity for chromosomes 13,14 and 21: implications for recombination between nonhomolgues and Robertsonian translocations. Nucleic Acids Res 16:1273-1284.

30. Choo, KH, Vissel, B, and Earle, E (1989) Evolution of α-satellite DNA on human acrocentric chromosomes. Genomics 5:332-344.

31. Cohen, MM, and Shaw, MV (1967) The association of acrocentric chromosomes in 1000 normal human male metaphase cells. Ann Hum Genet 31:129-140.

32. Cooke, P, and Curtis, DJ (1974) General and specific patterns of acrocentric association in parents of mongol children. Humangenetik 23:279-287.

33. Créau-Goldberg, N, Gegonne, A, Delabar, J, Cochet, C, Cabanis, M, Stehelin, D, Turleau, C, and de Grouchy, J (1987) Maternal origin of a de novo balanced t(21q21q) identified by ets-2 polymorphism. Hum Genet 76:396-398.

34. Curtis, DJ (1974) Acrocentric associations in mongol populations. Humangenetik 22:17-22.

35. Dagna Bricarelli, F, Pierluigi, M, Perroni, L, Grasso,M, Arslanian, A, and Sacchi, N (1988) High efficiency in the attribution of parental origin of non-disjunction in trisomy 21 by both cytogenetic and molecular polymorphisms. Hum Genet 79:124-127.

36. Daniel, A (1979) Single Cd band in dicentric translocations with one suppressed centromere. Hum Genet 48:85-92.

37. Daniel, A, and Lam-Po-Tang, PRLC (1976) Structure and inheritance of some heterozygous Robertsonian translocations in man. J Med Genet 13:381-388.

38. Daniel, A, Williams, K, and Lam-Po-Tang, PRLC (1980) Higher risk to D;G translocation carriers of tdic(13;21) as compared to tdic(14;21). J Med Genet 17:491-492.

39. Darlington, CD (1939) Misdivision and the genetics of the centromere. J Genet 37:341-364.

40. Darlington, CD (1940) The origin of iso-chromosomes. J Genet 39:351-361.

41. Davies, KE, Harper, K, Bonthron, D, Krumlauf, R, Polkey, A, Pembrey, ME, and Williamson, R (1984) Use of a chromosome 21 cloned DNA probe for the analysis of non-disjunction in Down syndrome. Hum Genet 66:54-56.

42. de Capoa, A, Ferraro, M, Menendez, F, Mostacci, C, Pelliccia, F, and Rocchi, A (1978) Ag staining of the nucleolus organizer (NO) and its relationship to satellite association. Hum Genet 44:71-77.

43. de Grouchy, J, and Turleau, C (1984) Clinical Atlas of Human Chromosomes. New York: John Wiley and Sons, p. 344.

44. de Kretser, DM, Burger, HG, Fortune, D, Hudson, B, Long, AR, Panesen, CA, and Taft, HP (1972) Hormonal, histological and chromosomal studies in adult males with testicular disorders. J Clin Endocr 35:392-401.

45. de la Chapelle, A (1982) How do human isochromosomes arise? Cancer Genet and Cytogenet 5:173-179.

46. de la Chapelle, A, and Stenstrand, K (1974) Dicentric human X chromosomes. Hereditas 76:259-268.

47. de la Chapelle, A, Wennstrom, J, Hortling, H, and Ockey,CH (1966) Isochromosome-X in man. Part I. Hereditas 54:260-276.

48. del Mazo, J, Castillo, AP, and Abrisqueta, JA (1982) Trisomy 21: Origin of non-disjunction. Hum Genet 62:316-320.

49. Devilee, P, Cremer, T, Slagboom, P, Bakker, E, Scholl, HP, Hager, HD, Stevenson, AFG, and Cornelisse, CJ (1986) Two subsets of human alphoid repetitive DNA show distinct preferential localization in the pericentric regions of chromosomes 13, 18 and 21. Cytogenet Cell Genet 41:193-201.

50. Devine, EA, Nolin, SL, Houck, GE, Jenkins, EC, and Brown, WT (1985) Chromosomal localization of several families of repetitive sequences by in situ hybridization. Am J Hum Genet 37:114-123.

51. Di Berardino, D, and Iannuzzi, L (1981) Chromosome banding homologies in Swamp and Murrah buffalo. J Hered 72:183-188.

52. Di Lernia, R, Riva, ML, Dalpra, L, and Ginelli, E(1980) Satellite associations and silver staining in a case of multiple G and D variants. Hum Genet 53:237-240.

53. Eiberg, H (1974) New selective giemsa technique for human chromosomes, Cd-staining. Nature 48:55.

54. Eklund, A, Simola, KOJ, and Ryynanen, M (1988) Translocation t(13;14) in nine generations with a case of translocation homozygosity. Clin Genet 33:83-86.

55. Engel, E, McGee, BJ, Hartmann, RC, and Engel de Montmollin, M (1965) Two leukemic peripheral blood stemlines during acute transformation of chronic myelogenous leukemia in a D/D translocation carrier. Cytogenet 4:157-170.

56. Evans, HJ, Buckland, RA, and Pardue, ML (1974) Location of the genes coding for 18S and 28S ribosomal RNA in the human genome. Chromosoma 48:405-426.

57. Evans, JA, Canning, N, Hunter, AGW, Martsolf, JT, Ray,M, Thompson, DR, and Hamerton, JL (1978) A cytogenetic survey of 14,069 newborn infants. III. An analysis of the significance and cytologic behavior of the Robertsonian and reciprocal translocations. Cytogenet Cell Genet 20:96-123.

58. Feinberg, AP, and Vogelstein, B (1983) A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal Biochem 132:6-13.

59. Feinberg, AP, and Vogelstein, B (1984) Addendum: A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal Biochem 137:266-267.

60. Ferguson-Smith, MA, and Handmaker, SD (1961) Observations on the satellited human chromosomes. Lancet 1:638-640.

61. Fernhoff, PM, Singh, DN, Hanson, J, Trusler, S, Dumont, CR, and Chen, ATL (1976) Association of D/D translocations with fetal wastage and aneuploidy: A report of four families. J Med Genet 13:389-393.

62. Ferraro, M, and Prantera, G (1988) Human NORs show correlation between transcriptional activity, DNase I sensitivity, and hypomethylation. Cytogenet Cell Genet 47:58-61.

63. Fraccaro, M, Ikkos, D, Lindsten, J, Luft, R, and Kaijser, K (1960) A new type of chromosomal abnormality in gonadal dysgenesis. Lancet II:1144.

64. Fraccaro, M, Maraschio, P, Pasquali, F, Tiepolo, L, Zuffardi, O, and Giarola, A (1973) Male infertility and 13/14 translocation. Lancet 1:488.

65. Gabriel-Robez, O, Ratomponirina, C, Croquette, M, Couturier, J, and Rumpler, Y (1988) Synaptonemal complexes in a subfertile man with a pericentric inversion in chromosome 21. Cytogenet Cell Genet 48:84-87.

66. Gallego, MS and Coco, R (1988) Non-disjunction and parental karyotypes. Medicina 48:147-155.

67. Galperin-LeMaitre, H, Hens, L, Kirsch-Volder, M, and Susanne, C (1977) Non-random association of trypsin-banded human acrocentric chromosomes. Hum Genet 35:261-268.

68. Galt, J, Boyd, E, Connor, JM, and Ferguson-Smith, MA (1989) Isolation of chromosome-21-specific DNA probes and their use in the analysis of nondisjunction in Down syndrome. Hum Genet 81:113-119.

69. Gardner, RJM, and Veale, AMO (1974) de novo translocation Down's syndrome:risk of recurrence of Down's syndrome. Clin Genet 6:160-164.

70. Garver, KL, Marchese, SG, Steele, MW, and Ketterer, DM (1982) Recurrence risk in 21q/21q translocation Down syndrome. J Pediatrics 100:243-245.

71. Goodpasture, C, Bloom, SE, Hsu, TC, and Arrighi, FE (1976) Human nucleolus organizers: The satellites or the stalks? Am J Hum Genet 28:559-566.

72. Gosden, JR, Gosden, CM, Lawrie, SS, and Buckton, KE (1979) Satellite DNA loss and nucleolar organiser activity in an individual with a de novo chromosome 13, 14 translocation. Clin Genet 15:518-529.

73. Gosden, JR, Lawrie, S, and Sevanez, H (1978) Ribosomal and human homologous repeated DNA distribution in the orangutan (Pongo pygmaeus). Comparison with the distribution of these DNAs in the other species of the Hominidae. Cytogenet Cell Genet 21:1-70.

74. Gosden, JR, Lawrie, SS, and Gosden, CM (1981) Satellite DNA sequences in the human acrocentric chromosomes:Information from translocations and heteromorphisms. Am JHum Genet 33:243-251.

75. Grasso, M, Giovannucci Uzielli, ML, Pierluigi, M, Tavellini, F, Perroni, L, and Dagna Bricarelli, F (1989) Isochromosome not translocation in trisomy 21q21q. Hum Genet 84:63-65.

76. Grell, RF and Valencia, JI (1964) Distributive pairing and aneuploidy in man. Science 145:66-67.

77. Guanti, G, Zuffardi, O, and Tiepolo, L (1980) rDNA levels in infertile male carriers of Robertsonian translocations. Cytogenet Cell Genet 27:162-167.

78. Hall, BD (1985) Letter to the editor: recurrence risk in de novo 21q21q translocation Down syndrome. Am J Med Genet 22:417-418.

79. Hamers, AJH, Vaes-Peeters, GPM, Jongbloed, RJE, Millington-Ward, AM, Meijer, H, de Die-Smulders, CEM, and Geraedts, JPM (1987) On the origin of recurrent trisomy 21:determination using chromosomal and DNA polymorphisms. Clin Genet 32:409-413.

80. Hamerton, JL (1968) Robertsonian translocation in man: evidence for prezygotic selection. Cytogenetics 7:260-276.

81. Hamerton, JL (1971) Human cytogenetics. New York and London: Academic Press, pp. 276-285.

82. Hamerton, JL, Canning, N, Ray, M, and Smith, J (1975) A cytogenetic survey of 14,069 newborn infants. I. Incidence of chromosomal abnormalities. Clin Genet 8:223-243.

83. Hamerton, JL, Giannelli, F, and Carter, CO (1963) A family showing transmission of a D/D reciprocal translocation and a case of regular 21-trisomic Down's syndrome. Cytogenetics 2:194-207.

84. Hanahan, D (1983) Studies on transformation of Escherichia coli with plasmids. Mol Biol 166:557-580.

85. Hansson, A (1975) Compensatory mechanisms in the satellite association patterns of individuals with Robertsonian translocations. Hereditas 81:101-112.

86. Hansson, A, and Mikkelsen, M (1978) The origin of the extra chromosome 21 in Down syndrome. Studies of fluorescent variants and satellite association in 26 informative families. Cytogenet Cell Genet 20:194-203.

87. Hara, Y, and Sasaki, M (1975) A note on the origin of extra chromosomes in trisomies 13 and 21. Proc Japan Acad 51: 295-299.

88. Harbinson, M, Hassold, T, Korbryn, C, and Jacobs, PA (1988) Molecular studies of the parental origin and nature of human X isochromosomes. Cytogenet Cell Genet 47:217-222.

89. Harris, DJ, Hankins, L, and Begleiter, ML (1979) Reproductive risk of t(13q14q) carriers: Case report and review. Am J Med Genet 3:175-181.

90. Hassold, T, Chen, N, Funkhouser, J, Jooss, T, Manuel, B, Matsuura, J, Matsuyama, A, and Wilson, C (1980) A cytogenetic study of 1000 spontaneous abortions. Ann Hum Genet 44:151-178.

91. Hassold, T, Jacobs, PA, and Pettay, D (1987) Analysis of nucleolar organizing regions in parents of trisomic spontaneous abortions. Hum Genet 76:381-384.

92. Hecht, F (1976) The nonrandomness of human chromosome abnormalities. In E. Hook and I. Porter (eds): Population Cytogenetics. New York: Academic Press, pp. 237-250.

93. Holden, JJA, Hough, MR, and White, BN (1989) Isochromosome formation by sister chromatid exchange. Karyogram 1:15.

94. Hook, EB (1978a) Monitoring human mutations and considerations of a dilemma posed by an apparent increase in one type of mutation rate. In N.E. Morton and C.S. Chung (eds): Genetic Epidemiology. New York: Academic Press.

95. Hook, EB (1978b) The ratio of de novo unbalanced translocation to 47, trisomy 21 Down syndrome. A new method for human mutation surveillance and an apparent recent change in mutation rate resulting in humn interchange trisomies in one jurisdiction. Mutation Res 52:427-439.

96. Hook, EB (1981) Unbalanced Robertsonian translocations associated with Down's syndrome or Patau's syndrome: Chromosome subtype, proportion of inherited, mutation rates and sex ratio. Hum Genet 59:235-239.

97. Hook, EB, and Albright, SG (1981) Mutation rates for unbalanced Robertsonian translocations associated with Down syndrome. Evidence for a temporal change in New York State live births 1968-1977. Am J Hum Genet 33:443-454.

98. Hook, EB, Schreinemachers, DM, Willey, AM, and Cross,PK (1983) Rates of mutant structural chromosome rearrangements in human fetuses: Data from prenatal cytogenetic studies and associations with maternal age and parental mutagen exposure. Am J Hum Genet 35:96-109.

99. Hook, EB, Schreinemachers, DM, Willey, AM, and Cross,PK (1984) Inherited structural cytogenetic abnormalities detected incidentally in fetuses diagnosed prenatally: Frequency, parental age associations, sex ratio trends, and comparisons with rates of mutation. Am J Hum Genet 36:422-443.

100. Horsthemke, B, Greger, V, Becher, R, and Passarge, E (1989) Mechanism of i(6p) formation in retinoblastoma tumor cells. Cancer Genet Cytogenet 37:95-102.

101. Hsu, LY (1986) Prenatal diagnosis of chromosome abnormalities. In: Genetic Disorders and the Fetus. Milunsky, A (ed). Plenum Publishing Corporation, New York. p. 115-183.

102. Hsu, LYF, Paciuc, S, David, K, Cristian, S, Moloshok, R,and Hirschhorn, K (1978) Number of C-bands of human isochromosome Xqi and relation to 45, X mosaicism. J Med Genet 15:222-226.

103. Hsu, TC (1979) Human and Mammalian Cytogenetics. An Historical Perspective. New York: Springer-Verlag.

104. Iannuzzi, L, Di Berardino, D, Gustavsson, I, Ferrara, L, and Di Meo, GP (1987) Centromeric loss in translocations ofcentric fusion type in cattle and water buffalo. Hereditas 106:73-81.

105. Ing, P (1975) Spatial relationships among human metaphase chromosomes: Analysis of the distances between chromosomes and patterns of satellite associations in normal subjects. Doctoral Thesis, Indiana University, Indianapolis, Indiana.

106. Ishikiriyama, S, and Niikawa, N (1984) Origin of extra chromosome in Patau syndrome. Hum Genet 68:266-268.

107. Jackson-Cook, CK (1985) The Extra Chromosome in Trisomy 21: Its Parental Origin and Etiology. Dissertation Medical College of Virginia, Richmond.

108. Jackson-Cook, CK (1990) Personal Communication.

109. Jackson-Cook, CK, Flannery, DB, Corey, LA, Nance, WE, and Brown, JA (1985) Nucleolar organizer region variants as a risk factor for Down syndrome. Am J Hum Genet 37:1049-1061.

110. Jackson-Cook, CK, Nance, WE, and Brown, JA (1988) Role of the Double Nucleolar Organizer Region (dNOR) Variant in the Etiology of Trisomy 21. In A. Daniel (ed): The Cytogenetics of Mammalian Autosomal Rearrangements. New York: Alan R. Liss, Inc., pp. 697-721.

111. Jacobs, PA (1981) Mutation rates of structural chromosomes rearrangements in man. Am J Hum Genet 33:44-54.

112. Jacobs, PA, Buckton, KE, Christie, S, Morton, N, and Matthew, D (1974) A family with two translcations and a polymorphism involving chromosome 14. J Med Genet 11:65-68.

113. Jacobs, PA, and Mayer, M (1981) The origin of human trisomy:a study of heteromorphisms and satellite associations. Ann Hum Genet 45:357-365.

114. Jacobs, PA, Mayer, M, and Morton, NE (1976) Acrocentric chromosome associations in man. Am J Hum Genet 28:567-576.

115. Jacobs, PA, Mayer, M, and Rudak, E (1978) Structural chromosome abnormalities in Down syndrome: a study of two families. Cytogenet Cell Genet 20:185-193.

116. Jorgensen, AL, Bostock, CJ, and Bak, AL (1987) Homologous subfamilies of human alphoid repetitive DNA on different nucleolus organizing chromosomes. Proc Natl Acad Sci 84:1075-1079.

117. Jorgensen, AL, Kolvraa, S, Jones, C, and Bak, AL (1988) A subfamily of alphod repetitive DNA shared by the NOR-bearing human chromosomes 14 and 22. Genomics 3:100-109.

118. Jotterand-Bellomo, M, and van Melle, G (1981) Variability of the nucleolar organizer activity in human lymphocytes via Ag-staining. Hum Genet 59:141-147.

119. Juberg, RC, and Mowrey, PN (1983) Origin of nondisjunction in trisomy 21 syndrome: all studies compiled, parental age analysis, and international comparisons. Am J Med Genet 16:111-116.

120. Kajii, T, Niikawa, N, and Merotto, E (1976) Origin of trisomics in human spontaneous abortions. Clin Genet 10:360.

121. Kikuchi, Y, Olshi, H, Tonomura, A, Yamada, K, Tanaka, Y,Kurita, T, and Matsunaga, E (1969) Translocation Down's syndrome in Japan: Its frequency, mutation rate of translocation and parental age. Jap J Hum Genet 14:93-106.

122. King, WA, Linares, T, Gustavsson, I, and Bane, A (1980) Presumptive translocation type trisomy in embryos sired by bulls heterozygous for the 1/29 translocation. Hereditas 92:167-169.

123. Kirkels, VGHJ, Hustinx, ThWJ, and Scheres, JMJC (1980) Habitual abortion and translocation (22q;22q): Unexpected transmission from a mother to her phenotypically normal daughter. Clin Genet 18:456-461.

124. Kohno, S, and Sandberg, AA (1978) Ph1-positive CML in a 13;14 translocation carrier. Med Pediatr Oncol 5:61-64.

125. Krystal, M, D'Eustachio, P, Ruddle, F, and Arnheim, N (1981) Human nucleolus organizers on nonhomologous chromosomes can share the same ribosomal gene variants. Proc Natl Acad Sci 78:5744-5748.

126. Kurnit, DM (1979) Satellite DNA and heterochromatin variants: the case for unequal mitotic crossing over. Hum Genet 47:169-186.

127. Kurnit, DM, Neve, RL, Morton, CC, Bruns, GAP, Ma, NSF, Cox, DR, and Klinger, HP (1984) Recent evolution of DNA sequence homology in the pericentromeric regions of human acrocentric chromosomes. Cytogenet Cell Genet 38:99-105.

128. Kurnit, DM, Roy, S, Stewart, GD, Schwedock, J, Neve, RL,Bruns, GAP, Van Keuren, ML, and Patterson, D (1986) The 724 family of DNA sequences is interspersed about the pericentromeric regions of human acrocentric chromosomes. Cytogenet Cell Genet 43:109-116.

129. Larsen, RE, Dias, E, Flores, G, and Selden, JR (1979) Breeding studies reveal segregation of a canine Robertsonian translocation along Mendelian proportions. Cytogenet Cell Genet 24:95-101.

130. Larson, LM, Wasdahl WA, Saumur, JH, Coleman, ML, and Jalal, SM (1978) Trisomy 18 syndrome with an unusual karyotype: possible double isochromosome. J Med Genet 15:73-76.

131. Lau, Y-F, and Hsu, TC (1977) Variable modes of Robertsonian fusions. Cytogenet Cell Genet 19:231-235.

132. Laurie, DA, and Hultén, MA (1985) Further studies on bivalent chiasma frequency in human males with normal karyotypes. Ann Hum Genet 49:189-201.

133. Lewin, B (1983) Genes. New York: John Wiley and Sons, Inc.

134. Lindenbaum, RH, Hultén, M, McDermott, A, and Seabright, M (1985) The prevelance of translocations in parents of children with regular trisomy 21: a possible inter-chromosomal effect?. J Med Genet 22:24-28.

135. Long, SE (1977) Cytogenetic examination of preimplantation blastocysts of ewes mated to rams heterozygous for the Massey I (t1) translocation. Cytogenet Cell Genet 18: 82-89.

136. Long, SE (1978) Chiasma counts and non-disjunction frequencies in a normal ram and in rams carrying the Massey I (t1) Robertsonian translocation. J Reprod Fert 53:353-356.

137. Lurie, IW (1979) Down's syndrome with 45 chromosomes. Hum Genet 40:121-123.

138. Magenis, RE, Overton, KM, Chamberlin, J, Brady, T, and Lovrien, E (1977) Parental origin of the extra chromosome in Down's syndrome. Hum Genet 37:7-16.

139. Maniatis, T, Fritsch, EF, and Sambrook, J (1982) Molecular cloning: a laboratory manual. Cold Spring Harbor,:Cold Spring Harbor Laboratory.

140. Markovic, VD, Worton, RG, and Berg, JM (1978) Inheritance of silver-stained nucleolus organizer regions. Hum Genet 41:181-187.

141. Marsden, HB, MacKay, RI, Murray, A, and Ward, HE (1966) Down's syndrome with a familial D/D reciprocal translocation and a G/G chromosome. J Med Genet 3:56-58.

142. Martin, RH (1988) Cytogenetic analysis of sperm from a male heterozygous for a 13;14 Robertsonian translocation. Hum Genet 80:357-361.

143. Martinez-Castro, P, Ramos, MC, Rey, JA, Benitez, J, and Sanchez Cascos, A (1984) Homozygosity for a Robertsonian translocation (13q14q) in three offspring of heterozygous parents. Cytogenet Cell Genet 38:310-320.

144. Matsunaga, EI, and Tonomura, A (1972) Parental age and birth weight in translocation Down's syndrome. Ann Hum Genet, Lond 36:209-217.

145. Mattei, JF, Ayme, S, Mattei, MG, Gouvernet, J, and Giraud, F (1976) Quantitative and qualitative study of acrocentric associations in 109 normal subjects. Hum Genet 34:185-194.

146. Mattei, JF, Mattei, MG, Ayme, S, and Giraud, F (1979) Origin of the extra chromosome in trisomy 21. Hum Genet 46:107-110.

147. Mattei, MG, Mattei, JF, Ayme, S, and Giraud, F (1979) Dicentric Robertsonian translocations in man: 17 cases studied by R,C and N banding. Hum Genet 50:33-38.

148. McDermid, HE, Duncan, AMV, Higgins, MJ, Hamerton, JL, Rector, E, Brasch, KR, and White, B (1986) Isolation and characterization of an alpha-satellite repeated sequence from human chromosome 22. Chromosoma 94:228-234.

149. McIlree, ME, Price, WH, Court Brown, WM, Tulloch, WS, Newsan, JE, and MacLean, N (1966) Chromosome studies on testicular cells from 50 subfertile men. Lancet 2:69-71.

150. Meijer, H, Hamers, GJH, Jongbloed, RJE, Vaes-Peeters, GPM, van der Hulst, RRWJ, and Geraedts, JPM (1989) Distribution of meiotic recombination along nondisjunction chromosomes 21 in Down syndrome determined using cytogenetics and RFLP haplotyping. Hum Genet 83:280-286.

151. Melnyk, JH, Persinger, GW, Coss, J, and Melnyk, ML (1987) Down syndrome and nucleolar organizing regions. Am J Hum Genet 41:A134.

152. Mikelsaar, Av, Schmid, M, Krone, W, Schwarzacher, HG, and Schnedl, W (1977) Frequency of Ag-stained nucleolus organizer regions in the acrocentric chromosomes in man. Hum Genet 37:73-77.

153. Mikkelsen, M (1971) Down's syndrome. current stage of cytogenetic research. Humangenetik 12:1-28.

154. Mikkelsen, M, Basli, A, and Poulsen, H (1980) Nucleolus organizer regions in translocations involving acrocentric chromosomes. Cytogenet Cell Genet 26:14-21.

155. Mikkelsen, M, Poulsen, H, Grinsted, J, and Lange, A (1980) Non-disjunction in trisomy 21: study of cytogenetic heteromorphisms in 110 families. Ann Hum Genet 44:17-28.

156. Miller, DA, Breg, WR, Warburton, D, Dev, VG, and Miller, OJ (1978) Regulation of rRNA gene expression in a human familial 14p+ marker Chromosome. Hum Genet 43:289-297.

157. Miller, DA, Tantravahi, R, Dev, VG, and Miller, OJ (1977) Frequency of satellite association of human chromosomes is correlated with amount of Ag-staining of the nucleolus organizer region. Am J Hum Genet 29:490-502.

158. Miller, OJ, Miller, DA, Tantravahi, R, and Dev, VG (1978) Nucleolus organizer activity and the origin of Robertsonian translocations. Cytogenet Cell Genet 20:40-50.

159. Millington-Ward, Am, and Pearson, PL (1988) Use of restriction fragment length polymorphic probes in the analysis of Down's syndrome trisomy. Hum Genet 80:362-370.

160. Mirre, C, Hartung, M, and Stahl, A (1980) Association of ribosomal genes in the fibrillar center of the nucleolus: A factor influencing translocation and nondisjunction in the human meiotic oocyte. Proc Natl Acad Sci 77:6017-6021.

161. Moorhead, PS, Nowell, PC, Mellman, WJ, Battips, DH, and Hungerford, DA (1960) Chromosome preparations of leukocytes cultured from human peripheral blood. Exp Cell Res 20:613-616.

162. Morgan, R, Bixenman, H, and Hecht, F (1985) Human Chromosome variation with two Robertsonian translocations. Hum Genet 69:178-180.

163. Mori, MA, Huertas, H, Pinel, I, Giralt, P, and Martinez-Frias, ML (1985) Trisomy 13 in the child of two carriers of a 13/15 translocation. Am J Med Genet 20:17-20.

164. Morton, CC, Brown, JA, Holmes, WM, Nance, WE, and Wolf,B (1983) Stain intensity of human nucleolus organizer region reflects incorporation of uridine into mature ribosomal RNA. Exp Cell Res 145:405-413.

165. Morton, CC, Corey, LA, Nance, WE, and Brown, JA (1981) Quinacrine mustard and nucleolar organizer region heteromorphisms in twins. Acta Genet Med Gamellol 30:39-49.

166. Muller, Hj, Buhler, EM, Signer, E, Egli, F, and Stalder,GR (1972) Trisomy-18 syndrome caused by translocation or isochromosome formation. J Med Genet 9:462-467.

167. Neri, G, Serra, A, Campana, M, and Tedeschi, B (1983) Reproductive risks for translocation carriers: cytogenetic study and analysis of pregnancy outcome in 58 families. Am J Med Genet 16:535-561.

168. Niebuhr, E (1972) Dicentric and monocentric Robertsonian translocations in man. Humangenetik 16:217-226.

169. Nielsen, J, and Rasmussen, K (1976) Autosomal reciprocal translocations and 13/14 translocations: A population study. Clin Genet 10:161-177.

170. Nikolis, J, and Kekic, V (1986) De novo 21/21 translocation Down syndrome: Studies of parental origin of the translocation and acrocentric associations in parents. Hum Genet 73:127-129.

171. Nikolis, J, and Kekic, V (1988) Evidence for a compensatory mechanism regulating Ag-NOR activity in families with de novo 21;21 translocation Down syndrome. Cytogenet Cell Genet 47:197-200.

172. Nikolis, J, Kekic, V, and Diklic, V (1981) NORs and satellite associations in a family with 13/14 translocation. Hum Genet 59:342-344.

173. Nusbacker, J, and Hirschhorn, K (1968) Autosomal anomalies in man. Advances in Teratology 3:1-63.

174. Ohno, S, Trujillo, JM, Kaplan, WD, and Kinosita, R (1961) Nucleolus-organisers in the causation of chromosomal anomalies in man. Lancet 2:123-126.

175. Olson, SB, Magenis, RE, and Lovrien, EW (1986) Human chromosome variation: The discriminatory power of Q-band heteromorphism (variant) analysis in distinguishing between individuals, with specific application to cases of questionable paternity. Am J Hum Genet 38:235-252.

176. Palmer, CG, Conneally, PM, and Christian, JC (1969) Translocations of D chromosomes in two families: t(13q14q) and t(13q14q)+(13p14p). J Med Genet 6:166-173.

177. Paris Conference, 1971 (1972) Standardization in human cytogenetics. Cytogenet 11:313-362.

178. Patil, SR, and Lubs, HA (1971) Non-random association of human acrocentric chromosomes. Humangenetik 13:157-159.

179. Pellestor, F, Sele, B, and Jalbert, H (1987) Chromosome analysis of spermatozoa from a male heterozygous for a 13;14 Robertsonian translocation. Hum Genet 76:116-120.

180. Penrose, LS (1962) Paternal age in mongolism. Lancet 1: 1101.

181. Penrose, LS, Ellis, JR, and Dalhanty, JDA (1960) Chromosomal translocations in mongolism and in normal relatives. Lancet 2:409-410.

182. Pérez-Castillo, A, and Abrisqueta, JA (1978) Patau's syndrome and 13q21q translocation. Hum Genet 42:327-331.

183. Petersen, MB, Slaugenhaupt, SA, Lewis, JG, Warren, AC, Chakravarti, A, and Antonarakis, SE (1989) A genetic linkage map of 24 loci on human chromosome 21. Am J Hum Genet 45:A157.

184. Pfeiffer, RA, and Loidl, J (1982) Mirror image duplications of chromosome 21. Three new cases and discussion of the mechanisms of origin. Hum Genet 62:361-363.

185. Phelan, MC, Prouty, LA, Stevenson, RE, Howard-Peebles, PN, Page, DC, and Schwartz, CE (1988) The parental origin and mechanism of formation of three dicentric X chromosomes. *Hum Genet* 80:81-84.

186. Phillips, RB (1975) Inheritance of acrocentric association patterns. Hum Genet 29:309-318.

187. Polani, PE, Briggs, JH, Ford, CE, Clark, CM, and Berg,JM (1960) A mongol girl with 46 chromosomes. Lancet 1:721- 724.

188. Polani, PE, Hamerton, JL, Granelli, F, and Carter, CO (1965) Cytogenetics of Down's syndrome (mongolism). III Frequency of interchange trisomies and mutation rate of chromosome interchange. Cytogenet 4:193-206.

189. Priest, JH, Blackston, RD, Au, KS, and Ray, SL (1975) Differences in human X isochromosomes. J Med Genet 12:378-389.

190. Priest, JH, Blackston, RD, Pearse, LA, and Warren, ST (1988) Molecular evidence for true isochromosome 21q. Hum Genet 81:1-3.

191. Pulliam, LH, and Huether, CA (1986) Translocation Down syndrome in Ohio 1970-1981: Epidemiologic and cytogenetic factors and mutation rate estimates. Am J Hum Genet 39:361-370.

192. Ray, M, and Pearson, J (1979) Nucleolar organizing regions of human chromosomes. Hum Genet 48:201-210.

193. Redi, CA, Garagna, S, Hilscher, B, and Winking, H (1985) The effects of some Robertsonian chromosome combinations on the seminiferous epithelium of the mouse. J Embryol Exp Morph 85:1-19.

194. Redi, CA, Hilscher, B, and Winking, H (1988) Unscheduled labelling by 3H-thymidine in mouse pachytene spermatocytes and round spermatids of Robertsonian translocation carriers. Cell Molec Biol 34:149-156.

195. Rhoads, MM (1940) Studies of a telocentric chromosome in Maize with reference to the stability of its centromere. Genetics 25:483-513.

196. Robertson, WmRB (1916) Chromosome studies: Taxonomic relationships shown in the chromosomes of Tettigidae and Acrididae: V-shaped chromosomes and their significance in Acrididae,Locustidae, and Gryllidae: Chromosomes and variation. J Morph 27:179-331.

197. Robinson, JA (1973) Origin of extra chromosome in trisomy 21. Lancet 1:131-133.

198. Rockman-Greenberg, C, Ray, M, and Evans, JA (1982) Homozygous Robertsonian translocations in a fetus with 44 chromosomes. Hum Genet 61:181-184.

199. Rowley, JD, and Pergament, E (1969) Possible non random selection of D group chromosomes involved in centric-fusion translocations. Ann Genet 12:177-183.

200. Rudd, NL, Dimnik, LS, Greentree, C, Mendes-Crabb, K, and Hoar, DI (1988) The use of DNA probes to establish parental origin in Down syndrome. Hum Genet 78:175-178.

201. Ruvinsky, AO, Agulnik, SI, Agulnik, AI, and Belyaev, DK (1987) The influence of mutations on chromosome 17 upon the segregation of homologues in female mice heterozygous for Robertsonian translocations. Genet Res Camb 50:235-237.

202. Schmid, M, Krone, W, and Vogel, W (1974) On the relationship between the frequency of association and the nucleolar constriction of individual acrocentric chromosomes. Humangenetik 23:267-277.

203. Schmidt, R, Dar, H, and Nitowsky, H (1975) Origin of extra 21 chromosome in patients with Down syndrome. Ped Res 9:318.

204. Schmidt, R, and Nitowsky, HM (1977) Recurrence of apparent de novo 21/21 translocation trisomy in a sibship. J Pediatrics 90:841-842.

205. Schmutz, SM, and Pinno, E (1986) Morphology alone does not make an isochromosome. Hum Genet 72:253-255.

206. Schwartz, S, Palmer, CG, Yu, P-L, Boughman, JA, and Cohen, MM (1986) Analysis of translocations observed in three different populations. Cytogenet Cell Genet 42:53-56.

207. Schwarzacher, HG, Mikelsaar, AV, and Schnedl, W (1978) The nature of the Ag-staining of nucleolus organizer regions. Cytogenet Cell Genet 20:24-39.

208. Sen, NK (1952) Isochromosomes in tomato. Genetics 37: 227-236.

209. Southern, EM (1975) Detection of specific sequences among DNA fragments separated by gel electrophoresis. J Mol Biol 98:503-517.

210. Spence, JE, Buffone, GJ, Rosenbloom, CL, Fernbach, SD, Curry, MR, et al. (1987) Prenatal diagnosis of cystic fibrosis using linked DNA markers and microvillar intestinal enzyme analysis. Hum Genet 76:5-10.

211. Spinner, NB, Eunpu, DL, Schmickel, RD, Zackai, EH, McEldrew, D, Bunin, GR, McDermid, H, and Emanuel, BS (1989) The role of cytologic NOR variants in the etiology oftrisomy 21. Am J Hum Genet 44:631-638.

212. Stahl, A, Luciani, JM, Hartung, M, Devictor, M, Berge-LeFranc, JL, and Guichaoua, M (1983) Structural basis for Robertsonian translocations in man: Association of ribosomal genes in the nucleolar fibrillar center in meiotic spermatocytes and oocytes. Proc Natl Acad Sci 80:5946-5950.

213. Steinberg, C, Zackai, EH, Eunpu, DL, Mennuti, MT, and Emanuel, BS (1984) Recurrence rate for de novo 21q21q translocation Down syndrome: A study of 112 families. Am J Med Genet 17:523-530.

214. Stene, J (1970) Statistical inference on segregation ratios for D/G translocations, when the families are ascertained in different ways. Ann Hum Genet 34:93-115.

215. Stene, J, and Stengel-Rutkowski, S (1988) Genetic risks of familial reciprocal and Robertsonian translocation carriers. In A. Daniel (ed): The Cytogenetics of Mammalian Autosomal Rearrangements. New York: Alan R. Liss, Inc., pp.3-72.

216. Stewart, GD, Harris, P, Galt, J, and Ferguson-Smith, MA (1985) Cloned DNA probes regionally mapped to human chromosome 21 and their use in determining the origin of nondisjunction. Nucleic Acids Res 13:4125-4133.

217. Stewart, GD, Hassold, TJ, Berg, A, Watkins, P, Tanzi, R, and Kurnit, DM (1988) Trisomy 21 (Down syndrome): Studying nondisjunction and meiotic recomination by using cytogenetic and molecular polymorphisms that span chromosome 21. Am J Hum Genet 42:227-236.

218. Stewart, GD, Hassold, TJ, and Kurnit, DM (1988) Trisomy 21. Molecular and cytogentic studies of nondisjunction. Adv Hum Genet 17:99-140.

219. Syme, RM, and Martin, RH (1988) Meiotic segregation of sperm chromosomes in a man heterozygous for a 21;22 Robertsonian translocation. Am J Hum Genet 43:A124.

220. Syme, RM, and Martin, RH (1989) Meiotic segregation of sperm chromosomes in a man heterozygous for a 15;22 Robertsonian translocation. Am J Hum Genet 45:A107.

221. Tantravahi, R, Miller, DA, Dev, VG, and Miller, OJ (1976) Detection of nucleolus organizer regions in chromosomes of human, chimpanzee, gorilla, orangutan and Gibbon. Chromosoma 56:15-27.

222. Tanzi, RE, Haines, JL, Watkins, PC, Stewart, GD, Wallace, MR, Hallewell,R, Wong, C, Wexler, NS, and Conneally, PM (1988) Genetic linkage map of human chromosome 21. Genomics 3:129-136.

223. Taylor, EF, and Martin-DeLeon, PA (1981) Familial silver staining patterns of human nucleolus organizer regions (NORs). Am J Hum Genet 33:67-76.

224. Therman, E (1980) Human Chromosomes. Structure, Behavior, Effects. New York: Springer-Verlag.

225. Therman, E, Susman, B, and Denniston, C (1989) The nonrandom participation of human acrocentric chromosomes in Robertsonian translocations. Ann Hum Genet 53:49-65.

226. Therman, E, Trunca, C, Kuhn, E, and Sarto, GE (1986) Dicentric chromosomes and the inactivation of the centromere. Hum Genet 72:191-195.

227. Uchida, IA, and Freeman, VCP (1986) Trisomy 21 Down syndrome. II. Structural chromosome rearrangements in the parents. Hum Genet 72:118-122.

228. Van Dyke, DL (1988) Isochromosomes and interstitial tandem direct and inverted duplications. In A. Daniel (ed): The cytogenetics of mammalian autosomal rearrangements. New York: Alan R. Liss,Inc., pp. 635-665.

229. Verma, RS, Babu, A, Chemitiganti, S, and Dosik, H (1986) A possible cause of non-disjunction of additional chromosome 21 in Down syndrome. Mol Gen Genet 202:339-341.

230. Verma, RS, Peakman, DC, Robinson, A, and Lubs, HA (1977) Two cases of Down syndrome with unusual de novo translocation. Clin Genet 11:227-234.

231. Vianna-Morgante, AM, and Nunesmaia, HG (1978) Dissociation as probable origin of mosaic 45,XY,t(15;21)/46,XY,i(21q). J Med Genet 15:305-310.

232. Vianna-Morganti, AM, and Rosenberg, C (1986) Deletion of the centromere as a mechanism for achieving stability of a dicentric chromosome. Cytogenet Cell Genet 42:119-122.

233. Walker, S, and Harris, R (1962) Familial transmission of a translocation between two chromosomes of 13-15 group (Denver Classification). Ann Hum Genet 26:151-162.

234. Wandall, A (1989) Kinetochore development in two dicentric chromosomes in man. A light and electron microscopic study. Hum Genet 82:137-141.

235. Warburton, D (1985) Interlaboratory Communication.

236. Warburton, D (1987) De novo structural rearrangements at amniocentsis: outcome and non-random position of breakpoints. Am J Hum Genet 41:A145.

237. Warburton, D, Atwood, KC, and Henderson, AS (1976) Variation in the number of genes for rRNA among human acrocentric chromosomes: correlation with frequency of satellite association. Cytogenet Cell Genet 17:221-230.

238. Warren, AC, Slaugenhaupt, SA, Lewis, JG, Chakravarti, A, and Antonarakis, SE (1989) A genetic linkage map of 17 markers on human chromosome 21. Genomics 4:579-591.

239. Waxmann, SH, and Arakaki, DT (1966) Familial Mongolism by a G/G mosaic carrier. J Pediatr 69:274-278.

240. Waye, JS, Mitchell, AR, and Willard, HF (1988) Organization and genomic distribution of "82H" alpha satellite DNA. Evidence for a low-copy or single-copy alphoid domain located on human chromosome 14. Hum Genet 78:27-32.

241. Wenger, RD, Aldenhoff, P, and Sperling, K (1980) Activity of rRNA genes in cells of a patient with Down syndrome mosaic. Hum Genet 55:227-229.

242. Wennstrom, J, and Schroder, J (1973) A t(13q14q) family with the translocation and a Philadelphia chromosome in one member. Humangenetik 20:71-73.

243. Willard, HF (1985) Chromosome-specific organization of human alpha satellite DNA. Am J Hum Genet 37:524-532.

244. Wilroy, RS, Summit, RL, and Atnip, RL (1969) De-novo G-G-translocation Down's syndrome in two siblings. Lancet 2:438.

245. Wilroy, RS, Summit, RL, and Martens, P (1978) Reproduction in a woman with mosaicism. J Med Genet 15:406-407.

246. Wilson, JA (1971) Fertility in balanced heterozygotes for a familial centric fusion translocation, t(DqDq). J Med Genet 8:175-178.

247. Yip, M-Y, and Fox, DP (1981) Correlations between relatives for acrocentric association frequency. Hum Genet 59:245-249.

248. Yunis, JJ, Alter, M, Hook, EB, and Mayer, M (1964) Familial D-D translocation. Report of a predigree and DNA replication analysis. N Engl J Med 271:1133-1137.

249. Zakharov, AF, Davudov, AZ, Benjush, VA, and Egolina, NA (1982a) Genetic determination of NOR activity in human lymphocytes from twins. Hum Genet 60:24-29.

250. Zakharov, AF, Davudov, AZ, Benjush, VA, and Egolina, NA(1982b) Polymorphisms of Ag-stained nucleolar organizer regions in man. Hum Genet 60:334-339.

251. Zang, KD, and Back, E (1968) Quantitative studies on the arrangement of human metaphase chromosomes. I. Individual features in the association pattern of the acrocentric chromosomes of normal males and females. Cytogenetics 7:455-470.

252. Zankl, H, and Hahmann, S (1978) Cytogenetic examination of the NOR activity in a proband with 13/13 translocation and in her relatives. Hum Genet 43:275-279.

253. Zankl, H, and Zang, KD (1974) Quantitative studies on the arrangement of human metaphase chromosomes. IV. The association frequency of human acrocentric marker chromosomes. Humangenetik 23:259-265.

254. Zellweger, H, Abbo, G, and Cuany, R (1966) Satellite association and translocation mongolism. J Med Genet 3:186-189.

Appendix

Confidentiality of Records

I have been informed that all information obtained from this study will remain strictly confidential and will be stored in a closely monitored, locked file cabinet. No participant will be identified by name in any publication(s) resulting from this research.

Withdrawal

I reserve the right to withdraw from this study at any time and I will receive a copy of this consent form.

Signed:______________________________ Date:____________

______________________________ Date:____________

Witness:______________________________ Date:____________

If you have any further questions, please feel free to contact us.

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