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AN ABSTRACT OF THE THESIS OF Diane Dusenbery Waggoner
for the Master of Science in Biology presented
November 29, 1983.

Title: Origin of Human Trisomy 21 Mosaicism

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Down Syndrome is a human condition caused by an extra copy of a #21 chromosome. At least one to two percent of free (not translocated) trisomy 21 cases are mosaics, i.e., they have two or more distinct cell

lines. Usually, one cell line is 47,XX or XY,+21 while the other cell line is normal 46,XX or 46,XY.

The purpose of the study was to establish the etiologies of the separate cell lines by determining whether the zygote was trisomic or normal. Meiotic non-disjunction in the formation of a gamete could lead to a trisomic zygote; loss of a #21 chromosome during a later mitotic division could then lead to a chromosomally normal cell line. Alternatively, a mitotic error in a normal embryo can produce a trisomy 21 cell line.

Of the six previously published reports of the origin of autosomal mosaicism, one was of a phenotypically normal trisomy 21 mosaic woman in whom the trisomic cell line resulted from either a mitotic embryonic error, or from an error in meiosis II in her father. The other five cases, involving chromosomes other than #21, all resulted from errors during meiosis followed by mitotic loss of an extra chromosome.

This study examines the chromosomes of seven trisomy 21 mosaics, two trisomy 13 mosaics, and thirteen of their parents to determine the origin of the two cell lines in the mosaics. In addition, the chromosomes of the parents and affected children were examined in two families which each had two children with trisomy 21.

Two fluorescent chromosome banding techniques, Q- and R-banding, were used to delineate differences in

size and brightness in the short arm, stalk or satellite regions of the chromosomes 21 or 13. Any heteromorphisms revealed are normal, heritable variants of no phenotypic significance.

Three of the trisomy 21 mosaics had three different #21s in their trisomic cell line; they could have come only from trisomic zygotes. A fourth trisomy 21 mosaic had a chromosome in the trisomic cell line which was not present in the normal cell line; this could have arisen only from a trisomic zygote.

In two trisomy 21 cases, including one with no sign of Down Syndrome except transient neonatal leukemia, the trisomic cell line contained two copies of one of the mother's #21 chromosomes. This could have occurred either as the result of a maternal meiosis II error or as the result of a mitotic error in the embryo.

One case of 45,X/47,XX,+21 and both trisomy 13 cases had uninformative heteromorphisms.

Mosaicism was not established in the parents in the families with two children affected with Down Syndrome, but in both cases one child was the result of a maternal meiosis I error and one of a maternal meiosis II error. Only 19% of cases of non-familial trisomy 21 are the result of a meiosis II error. An excess of apparent meiosis II errors could be the result of undetected mosaicism in a phenotypically normal parent who had two

copies of a #21 in a germ cell line.

In summary, in four cases the trisomic cell line arose because of an error during meiosis in a parent (maternal meiosis I error in at least two of the four) followed by a mitotic loss of one #21 in an embryonic division. In two cases, the trisomic cell line arose as the result of either an error at maternal meiosis II or as a mitotic error in a normal embryo. If only the typical trisomy 21 mosaics are considered, the distribution of meiosis I and meiosis II errors is not inconsistent with a meiotic origin of the trisomic cell line followed by mitotic loss of the third #21 chromosome.

ORIGIN OF HUMAN TRISOMY 21 MOSAICISM

by

DIANE DUSENBERY WAGGONER

A thesis submitted in partial fulfillment of the
requirements for the degree of

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in
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1984

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PREFACE

My interest in human chromosomal mosaicism, the existence of more than one distinct cell line in the same individual, arose while working from 1978 to 1981 as a research assistant in Dr. Ellen Magenis' chromosome research laboratory at the Oregon Health Sciences University.

Dr. Magenis had a grant from the National Institute of Health to study chromosome misbehavior, including the origin of the extra chromosome in Down Syndrome (Trisomy 21). By 1977 she had published the results of 31 cases of trisomy 21. Both the parent and the stage of formation of the egg or sperm in which the error had occurred had been established by using a special stain which reveals heritable differences among #21 chromosomes.

The technique permits the identification of which parent contributed two #21 chromosomes and whether the child received one copy of each of the parental #21s (80% of all cases) or two copies of one of the parents' #21s (20% of all cases).

One of the cases I worked on in the laboratory involved an infant who had not only a typical Down cell

line (47,XY,+21), but also a normal cell line (46,XY). I became curious about which cell line represented the original cell at the time of fertilization. If the zygote had been normal, improper distribution of the two #21 chromosomes at an embryonic division could have resulted in a trisomy 21 cell line with two identical copies of one of the #21s. If, however, the zygote had been trisomic, as the result of a meiotic error in one of the parents, and if one of the #21s had been lost during an embryonic division, then a normal cell line could have been produced. In the later case, the trisomic cell line would have three different #21 chromosomes in the trisomic cell line in 80% of the cases.

My plan was to study at least ten chromosome mosaics and their parents to determine which cell line was the original and which was derivative. I also planned to study families which had more than one trisomy 21 child to look for evidence of mosaicism in one of the parents.

ACKNOWLEDGEMENTS

I thank Dr. Ellen Magenis for making it possible for me to do this research. She has long been interested in mosaicism, and I am grateful for the use of the facilities of her chromosome research laboratory and her efforts in obtaining blood samples.

I also thank Dr. Lester Newman and Jan Chamberlin for their interest and help, and Leland Allen for photographing Figures 4 and 5.

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CHAPTER I

INTRODUCTION

HISTORY OF DOWN SYNDROME AND ITS CYTOGENETIC BASIS

Down Syndrome is a condition that is present in about one of every seven hundred newborns. Its characteristics include: mental retardation; short stature; round, flat face; flat occiput; epicanthal folds; oblique palpebral fissures; flat nasal bridge; open mouth with furrowed, protruding tongue; short, broad hands; clinodactyly of the fifth finger; unusual finger, palm and foot print patterns; hyperflexibility; hypotonia; and congenital heart disease. It usually occurs sporadically, but is much more common among children born to older mothers. The incidence reaches 1% of all children born to women over forty years old.

The syndrome was described in English separately by Down and by Sequin in 1866. Down (1866) described a "Mongolian type of idiocy" which occurred in over 10% of the retarded patients whom he saw. His description included:

The face is flat and broad, and destitute of prominence. The cheeks are roundish, and extended laterally. The eyes are obliquely

placed....The tongue is long, thick, and is much roughened....The skin...is deficient in elasticity, giving the appearance of being too large for the body....The circulation is feeble.

Down believed that tuberculosis in the parents had broken down racial barriers to produce Mongols in the offspring of Caucasians, an example which furnished "some arguments in favor of the unity of the human species."

Sequin (1866) described:

...furfuraceous [scaly] cretinism with its...peeling skin; with its shortcomings of all the integuments, which give an unfinished aspect to the truncated fingers and nose; with its cracked lips and tongue, with its red, ectropic conjunctiva, coming out to supply the curtailed skin at the margin of the lids.

Fraser & Mitchell (1876) were the first to note advanced maternal age in what they called Kalmuck idiots.

Waardenburg (1932) was the first person to suggest that:

...one should study mongolism for its possible association with 'a chromosomal deficiency' through 'non-disjunction' or the reverse, 'chromosomal duplication'...my hypothesis has the advantage that it can be tested. It would, in addition, explain the potential effect of maternal age.

The presence of an extra chromosome in the fibroblasts in each of three boys with Down Syndrome was reported by Lejeune, et al. (1959a) in January of 1959.

Instead of 46 chromosomes, which had been reported by Tjio and Levan in 1956 as the normal human diploid number, there was an extra small, nearly telocentric chromosome indistinguishable from the other five small chromosomes usually seen in normal males. By March, Lejeune, et al. (1959b) reported on a total of nine cases. All had 47 chromosomes in their fibroblasts. The five boys had a total of six small telocentrics and the four girls had five. They proposed that the cause was non-disjunction during meiosis of a pair of the small chromosomes and suggested that such a mechanism could account for the increasing rate with maternal age because non-disjunction in *Drosophila* is strongly influenced by maternal age. They could not, however, discount the possibility that the supernumerary chromosome was a fragment from some other kind of aberration.

In April, Jacobs, et al. (1959) reported that bone marrow studies of six patients showed that all had 47 chromosomes. It was thought likely that they were trisomic for one of the smallest acrocentric autosomes as a result of missegregation during meiosis, but could not exclude the possibility that the chromosome was a supernumerary chromosome of unknown origin.

Also in April, Ford, et al. (1959a) reported a patient with signs of both Klinefelter's syndrome and

mongolism who had 48 chromosomes in bone marrow specimens. He had a normal set of twenty-three pairs, including two X chromosomes, a Y chromosome, and an extra acrocentric chromosome.

In September, Book, et al. (1959) reported their cytological observations of both bone marrow and skin of a male and a female mongoloid. Both tissue types had 47 chromosomes and the extra chromosome was considered to be the size of a #21, but they did not definitely decide it was a third homologue rather than a structurally different chromosome.

April, 1959 also saw the publication of a case of chromosome mosaicism, though not in Down Syndrome. In Ford, et al. (1959b) the results of a bone marrow study of a man with Klinefelter's syndrome were interpreted as demonstrating both 47,XXY and 46,XX cells. It was supposed that the 46 count cell line arose through loss of the Y chromosome by mitotic non-disjunction and that it could have a selective advantage over the abnormal cells.

By 1961, it was agreed that the extra chromosome in mongolism was an extra copy of a G-group chromosome. That same year, mosaicism was first described in Down Syndrome. In May, Clarke, et al. (1961) described a two-year-old child whose parents were twenty-six years

old when she was born, and who had normal intelligence in spite of a somewhat mongoloid appearance. Cytological examination revealed that she was chromosomally mosaic. At 22 months, all eleven cells analyzed from a peripheral blood sample were normal 46,XX, while skin cultures at 18 and 22 months showed 32% and 38% trisomy 21 cells. They suggested that the mosaicism could have originated in either a trisomic zygote through loss of the extra chromosome by non-disjunction or anaphase lag or in a normal zygote in which non-disjunction, after the first cleavage division, of a chromosome 21 led to a 47 chromosome cell population and an inviable 45 chromosome cell. In any case, the combination of chromosome and clinical findings (mongol palmprint, normal intelligence, and Brushfield spots) implied that the mosaicism arose at an early development stage. They considered that the neuroectodermal origin of the iris where the Brushfield spots arise suggested nervous system involvement in the abnormal phenotype.

Clarke, et al. (1963) found that proportions of trisomy 21 and normal cells appeared to change with age. At age four she had 17% trisomic bone marrow and 14% trisomic blood cells.

In July, 1961, Fitzgerald and Lycette (1961a; 1961b) presented the case of a severely retarded fifty-

one-year-old mongol who lacked some typical mongoloid traits and who had been born to a thirty-eight-year-old woman. Of 100 peripheral blood cells, 42 had 46 chromosomes. Analyzed cells showed five small acrocentrics, typical of a normal male. 53 of the cells had six small acrocentrics, typical of male mongols, and five of the cells had 48 chromosomes, including seven small acrocentrics. It was thought most probable that this mosaic resulted from non-disjunction in the second or later cleavage division of a zygote which was trisomic for chromosome 21.

Mosaicism in the mother of a mongol child was reported by Blank, et al. (1962). The mother had some physical features of mongolism and an I.Q. of 60. Her child, born when she was twenty-eight, was a classical mongol with 47 chromosomes with an extra 21 or 22 in each of her peripheral blood cells. In the mother, however, 85% of the cells had 46 chromosomes with a typical female pattern, while 15% had 47 chromosomes with an extra chromosome in the 21-22 group. Because the mother was born when her mother was 40 years old, it was suggested that the propositus originated from a trisomic embryo. They suggested that some or all of her oocytes could contain 47 chromosomes and through secondary non-disjunction produce gametes with 24 in

addition to gametes with the normal 23 chromosomes.

Mosaicism in the normal 19-year-old mother of two non-twin mongoloid boys was reported by Smith, et al. (1962). Blood cultures showed that 27% of her cells were trisomic, while six of eight skin tissue culture cells were trisomic.

These early reports of chromosomal mosaicism in mongolism reflect the wide range of phenotypes expressed by people who have a mixture of normal and 47,+21 cells; fully affected with typical features, moderately affected with a range of physical and intellectual stigmata, and apparently normal who are usually discovered only after having more than one affected child.

In 1961, several geneticists, in a letter to Lancet (Allen, et al. 1961), urged the abandonment of the term "mongolism" because it was misleading, ambiguous, and embarrassing. Suggested replacements included "Down's Syndrome" and "trisomy 21 anomaly." The signers included J. A. Book, C. O. Carter, C. E. Ford, J. Lejeune, L. S. Penrose, P. E. Polani, Curt Stern, and R. Turpin. There is a current effort to drop the possessive from eponyms so that the most appropriate terms may now be "Down Syndrome" and "Trisomy 21."

INCIDENCE OF DOWN SYNDROME AND MOSAIC DOWN SYNDROME

Estimates of the incidence of mosaicism in Down Syndrome range from 1% to 5%. Smith and Berg (1976), in the second edition of Down's Anomaly, use Richard's (1969) figure of 2%, which was based on finding 51 mosaics among 2,466 patients in a total of 19 studies published from 1964-1967. Sach's (1971) book, Trisomy G/Normal Mosaicism includes a literature survey of patients with clinical signs of Down Syndrome. The percentage of mosaics in ten studies (1963-1969) each of more than 70 patients was 2.6% (32/1234). The rate for the five studies of at least 100 patients was 2.7%. Three studies of patients with doubtful signs of Down Syndrome discovered that 20/552 (3.6%) were mosaic. Hook (1981) summarized six studies from four continents, each of which had been published from 1970 to 1979 and included more than 300 Down Syndrome patients. The percentage of mosaics ranged from 1.6-4.4% per study, while the proportion of translocations had a narrower range of 3.1-5.6%.

Table I summarizes nine studies of at least 175 patients each of which was published from 1980 to 1983. The overall percentage of mosaics was 1.6% with seven of the studies ranging from 1.3-1.8%, while one study had

TABLE I
INCIDENCE OF MOSAICISM IN DOWN SYNDROME

Reference	Location	Incidence of D.S.	#D.S.	#Mos.	%Mos.	#Trans.	%Trans.
Adeyokunna, 1982	Nigeria	1/865	386	6	1.6	9	2.3
Giuffre', 1981	Italy		204	3	1.5	4	2.0
Gripenberg, 1980	Finland		305	4	1.1	15	4.9
Koulischer, 1980	Belgium	1/813	268	1	0.4	7	2.6
Lindsten, 1981	Sweden	1/780	1154	21	1.8	60	5.2
Moric'-Pet. 1981	Serbia	1/646	601	13	2.2	31	5.2
Owens, 1983	England	1/719	175	3	1.7	11	6.3
Zergollem, 1981	Croatia	1/820	472	6	1.3	17	3.6
Zizka, 1980	Czechoslovakia		<u>300</u>	<u>5</u>	<u>1.7</u>	<u>18</u>	<u>6.0</u>
	TOTAL:		3865	62	1.6	172	4.5

0.4% (1/268) and one was 2.2% (13/6701). Koulischer and Gillerot (1980) found only one mosaic among the 268 Down Syndrome patients they studied even though they examined 20 cells per patient in nearly every case. They suggest that their low incidence may reflect the fact that all their patients were newborns, some of whom (with non-mosaic trisomy 21) died shortly after birth. If the study had been done on older infants or children the mosaic proportion would have increased. This implies that mosaics--by being somewhat less severely affected--may tend to have longer life spans than full trisomy 21 cases.

An increase in the incidence of mosaicism with increasing age of the patients studied may be an explanation for the high number of mosaics detected by Krishna Murthy (1981). The study was conducted in Bombay, where medical services for fully affected Down Syndrome patients are probably less available than in Europe or the United States. Of 113 patients who had trisomy 21, nine of 78 (11.5%) who were born to mothers thirty or younger and three of 35 (8.6%) of those born to mothers over thirty were mosaic. The patients ranged in age from newborn to sixteen years old, except for one (mosaic) 65-year-old woman. At least 30 and up to 100 cells were examined for each patient and the proportion

of trisomic cells ranged from 15% (in the 65 year old) to 70%. It seems likely then that these truly were mosaic patients. An additional explanation for the high number of mosaics may be that patients with doubtful signs of Down Syndrome were included in the study. Thirteen of the original 126 cases (10.3%) were chromosomally normal.

The incidence of mosaicism in fetuses examined early in pregnancy (16 to 17 weeks) could be somewhat less than that seen in newborns if the mosaics are less likely to be aborted. The numbers are too small to be statistically significant, but only one mosaic has been reported among 136 trisomy 21 amniocenteses (Table II). Only studies in which mosaicism would have been mentioned were included; most did not indicate how many cells were examined. In all but eleven cases, the indication for amniocentesis was maternal age; the one mosaic trisomy 21 fetus was born to a mother who was 35 to 39 years old. Among spontaneous abortions, no mosaics were found in the 64 cases of trisomy 21 from four studies in which mosaicism was mentioned (Table III).

Hsu (1982) reported that among 62,279 amniocenteses there were 89 cases of true autosomal chromosomal

TABLE II

INCIDENCE OF MOSAICISM IN
SECOND TRIMESTER FETUSES
IN AMNIOCENTESES

Reference	Indication					
	Maternal Age			Other		
	#Screened	#Trisomy 21	#Mosaics	#Tested	#Trisomy 21	#Mosaic Trisomy 21
Crandall, 1980	1887	19	0	613	1	0
Cruikshank, 1983	665	13	0	258	0	0
Daniel, 1982	2227	29	0	773	0	0
Golbus, 1979	2404	36	1 ^a	596	3	0
Manganiello, 1979	149	2	0	103	1	0
NICHD, 1978	494	1	0	456	3	0
Simpson, 1976	466	9	0	447	3 ^b	0
Squire, 1982	1297	16	0	739	0	0
TOTALS:	9589	125	1	3985	11	0

^a maternal age 35-39 year^b one mother subsequently found to be trisomy 21 mosaic

TABLE III

INCIDENCE OF SINGLE AUTOSOMAL TRISOMY MOSAICISM
IN SPONTANEOUS ABORTIONS

Reference	#Aneu- ploids	Minimum #Cells Examined	#Tri. 21	#Mosaic Tri. 21	#Other Acrocent.	#Mosaics cen. Tri.	#Non-acro- cen. Tri.	#Mosaic	%Mosaics
Hassold 80									
Hassold 82	386	10	42	0	110	0	234	18	7.7
Meulenbroek/ Geraedts 82	27	5	4	0	10	0	13	1	7.7
Niikawa 77 Kajii 80	138	10	12	0	52	1 ^a	74	0	0.0
Stene/War- burton 81	254				79 ^b	1 ^b	175	19	10.9
Warburton 78	<u>(101)</u>	<u>10</u>	<u>(6)</u>	<u>0</u>	<u>(21)</u>	<u>(0)</u>	<u>(74)</u>	<u>(9)</u>	<u>(12.2)</u>
TOTALS:	805		58	0	251	2	496	38	

2/309 = 0.65% mosaic acrocentric trisomies
 38/496 = 7.7% mosaic non-acrocentric trisomies

^a Karyotyped on seventh passage (mosaic trisomy 22)

^b Stene (1981) referred to all acrocentrics as a group

mosaicism (0.14%). These included 21 cases of trisomy 21 mosaicism, or one per 2966 amniocenteses.

Estimates of the incidence of mosaicism are complicated by the difficulties inherent in detecting mosaicism. There are important requirements for such detection: 1) the tissue type sample must contain both cell lines, 2) the sample must be obtained at a time in the individual's life when both cell types are present in the examined tissue, and 3) enough cells must be examined to determine the existence of the separate cell lines when one line makes up only a small percentage of all cells. It is clear that current estimates of the occurrence of mosaicism are lower than its true incidence.

The most common tissues used in cytogenetic analysis, fibroblasts and lymphocytes, often have different proportions of the two cell types in the same mosaic individual. For instance, in the Down mosaic case of Clarke, et al. (1963) it was found that 34% of the patient's fibroblasts--but only 13% of her leucocytes--were trisomic for chromosome 21. Lindsten, et al. (1962) studied a two year old who had normal intelligence but some physical characteristics of Down Syndrome. Two blood samples averaged 63% trisomy 21 cells (73% and 53%), while three skin samples averaged 29% trisomic cells (50%, 12% and 28%). Ridler, et al.

(1965) reported on a severely retarded fifty-year-old man who had Down Syndrome. Only one of 106 blood cells was trisomic but skin cultures had 75% trisomic cells. Taylor (1970) studied the phenotypically normal mother of a Down Syndrome child. Blood and skin cells were 6% trisomic, but ovarian fibroblasts were 88% trisomic from the right ovary and 92% trisomic from the left.

Penrose and Smith (1966) suggested that trisomic cells often appear twice as frequently in the fibroblasts as in the leucocytes. Richards (1969) however, showed that skin cultures are usually done on a biased sample of mosaics: those with a low proportion of trisomic cells in their blood. The difference in percentages of trisomic cells in blood and skin would probably not be as great in an unselected sample of mosaics.

Clarke, et al. (1961; 1963), in noting the normal blood cells in their mosaic patient, proposed that selective proliferation of normal cells in tissues which undergo frequent mitosis, such as bone marrow, could lead to an increased percentage of normal cells in vivo. Taylor (1970) discounted in vitro cell selection in blood cultures because samples studied from 48-hour cultures, after the first division, are similar to those done at 72 hours, the standard time for blood cultures.

However, fibroblasts in tissue culture may undergo cell selection in vitro. Taylor (1970) divided a skin biopsy from a patient into two samples. One sample showed selection for normal cells while the other one selected for trisomic cells. Ridler, et al. (1965) found 94% (29 of 31) trisomic cells in one skin sample from their patient. Six months later they set up two separate cultures from a second skin biopsy. The one grown for 17-19 days had 42% trisomy (16 of 38 cells) while that grown for 24 days had 90% trisomy (25 of 28 cells). It therefore seems that extrapolation from the results of skin cultures is of limited usefulness in determining the percentage of mosaicism in in vivo fibroblasts.

"Disappearing mosaicism" was the term used by LaMarche, et al. (1967) to describe their patient who had trisomy 18 in 90% (62/70) of her blood cells at birth, but no trisomic cells in studies of 35 cells each that were done at 10 and 12 months. A bone marrow preparation at one month (30 cells) and skin cultures at three and four months (62 cells total) also showed no trisomic cells.

Taylor (1968, 1970) followed eight mosaic trisomy 21 children for up to five years. Three children, who had 15-35% trisomic cells at first examination (new-born to two months old), had only 1-2% trisomic cells at

ages two to five years. Two children, who had about 80% trisomic cells when first studied at six days and one month, had about 50% trisomic cells at 18 months and four years. Two children, with about 75% trisomic cells at four days and three months, later had about 95% trisomic cells at two years. One child's trisomic cells fluctuated between 0 and 35% in eight studies done between four and 32 months of age. Of the eight children, three had 0-2% trisomic cells by 20-30 months of age and two had 97-98% trisomic cells by three and twelve months. Unless large numbers of cells had been examined, mosaicism likely would have been missed in five of these eight children if they had first been studied after their first or second birthday. Taylor concluded that rapid cell selection for either normal or trisomic cells occurs during the first two to three years of life. In contrast to the rapid changes in the proportions of cell lines in children were the results for three adults (16 to 32 years) who were followed eight months to four years. The 32 and 33 year olds initially had 4% trisomic cells and the sixteen year old had 18% trisomic cells. During the course of the study, none changed by more than a few percentage points.

Wilson, et al. (1980) summarized their studies of a girl with minimal signs of Down Syndrome whom they

followed for nine years. At six weeks of age, 32% of 100 blood cells had trisomy 21; at seven months, 18%. Four studies from 11 months to four years showed 4-9% trisomic cells, while at nine years, only three of 200 cells (1.5%) were trisomic. At 2-1/2 months, 3-4% of skin and bone marrow cells were trisomic.

Another problem in the ascertainment of mosaicism is the interpretation of a low number of either normal or trisomic cells. Penrose (1967) used a criterion for mosaicism in which the trisomic cell line was at least 9% but not more than 91% of all cells examined. These arbitrary limits continue to be quoted (Sachs, 1971; Smith and Berg, 1976; Hamerton, 1981), but they should be modified as techniques of chromosome analysis improve.

When there is a Down Syndrome phenotype and there are any trisomic cells in addition to normal cells, mosaicism probably exists. The more difficult problem is that of hypodiploidy, in which only a few cells have two #21 chromosomes, because chromosomes can be lost during sample preparation. The frequency of artifactual hypodiploidy will vary within and among different laboratories. Knowledge of the laboratory background rate is important in analyzing the finding of normal cells in a Down Syndrome patient. The problem is exacerbated in

Down Syndrome because small chromosomes such as #21 are more likely to be "randomly" lost. Smith and Elliott (1980) found that G(chromosomes 21 and 22) and E(16, 17, and 18) group chromosomes were much more likely to be missing from hypomodal cells than were other chromosomes ($P < .001$). They had a very high rate of hypomodality (8.1% of 3,175 cells from 100 consecutive patients) and their background rate for loss of a G-group chromosome was 2.5%.

Ford and Lester (1982) studied the attachment of chromosomes to the spindle of metaphase cells which had not been treated with colchicine or hypotonic. "Displaced" chromosomes, further from the spindle than the length of a chromosome 22, were found in 21% of metaphases. Of 382 displaced autosomes, 16 were displaced 0 to 17 times, two (chromosomes 15 and 18) were displaced 25 and 23 times, and four (chromosomes 16, 19, 21, and 22) were displaced 50-55 times. This confirmed the hypothesis that the smaller the chromosome, the more likely it is to be displaced ($P < .0001$).

Both studies indicate the propensity of chromosome 21 to be lost during in vitro mitosis. It is therefore important that suspected mosaics with a low percentage of normal cells be studied very carefully.

Recent improvements in chromosome staining, however, make it possible to differentiate truly random loss of a #21 chromosome from a trisomic cell line from a consistent loss of a specific #21. The fluorescent dye quinacrine reveals heteromorphisms of the acrocentric chromosomes 13, 14, 15, 21, and 22, of the centromeric regions of 3 and 4, and of the long arm of the Y (Caspersson, et al. 1971). (See Figure 1 for a sample quinacrine karyotype.) In the acrocentrics the differences include ranges in size and brightness of the centromeric, short arm and satellite regions (Figure 2). The short arms contain repetitive "satellite " DNA (Gosden, et al. 1975), while the DNA composition of the chromosome satellites is not known, although they apparently contain neither ribosomal nor satellite (repetitive) DNA (Gosden, et al. 1978). There is also a range of length in the non-staining stalk region between the short arm and satellite. Differences in the stalk region, which contains 18s and 28s ribosomal DNA (Evans, et al. 1974), may be seen through R-banding (reverse-banding) which also reveals differences in brightness and size. These heteromorphisms are normal variants and have no phenotypic significance. They often, however, permit the identification of individual homologues of acrocentric pairs. When all the #21s are different, and

Figure 1. Q-banded karyotype of a normal woman (46,XX). Arrows indicate heteromorphous regions of chromosomes 3 and 13. (Courtesy of Diane Tomar, Cytogenetics Research Laboratory, Oregon Health Sciences University)

Figure 2. Ideogram of chromosome 21 illustrating satellite, stalk, short arm and centromere regions. Q(quinacrine)-banding reveals differences in brightness, size, and shape of the satellites and short arms. R(reverse)-banding reveals differences of size and brightness of the stalk region. [From Olson SB et al (1983) Am J Med Genet 15:47-55]

the same one is not present in the disomic line, then in a large series of cells, even with a low percentage of normal cells, random loss of #21s may be ruled out and mosaicism may be assumed. A statistical analysis may be made of the probability of always getting the same homologues if loss were truly random. Thus, Penrose's original limits of mosaicism of 9-91% for either cell line can now be modified in some cases.

Hook (1977) has devised tables to determine confidence levels of the percentage of mosaicism that may be excluded when a number of cells have identical karyotypes. The fact remains, however, that mosaicism can never be ruled out. It may always be possible that if more cells were studied or from another tissue or at a different time, a second cell line might be discovered.

ORIGIN OF DOWN SYNDROME AND MOSAICISM

Mosaic Down Syndrome may be the result of an originally trisomic zygote (47,+21) or a normal zygote (46 chromosomes), with subsequent mitotic loss or gain of a chromosome 21, respectively, to lead to the second cell line. If the zygote is trisomic and the normal cell line results from loss of one #21 during mitosis, then there should be a maternal age effect and the chromosomes of the trisomic cell line will resemble those seen in full trisomy 21, usually with three detectably different #21s. If, however, the zygote is normal and the trisomic cell line results from mitotic non-disjunction in an embryonic division, then the trisomic cell line will have two identical #21s.

Richards (1974) estimated that 19% of mosaics come from normal zygotes and the remainder come from trisomic zygotes, based on maternal age effect. Penrose had observed that the mean age of mothers of mosaics is somewhat reduced in the direction of normal controls while there is a strong maternal age effect in trisomy 21. Richards divided the difference between the mean maternal age of 108 each full trisomics (33.3 years) and that of mosaics (32.1 years) by the difference between the trisomics and normal controls (27.0 years) to give the 19% figure. Analysis of 15

Down controls (mean maternal age 34.7 years), mosaic parents (31.3 years), and normal controls (28.5 years), suggested that 53% of the phenotypically normal mosaic parents were derived from normal zygotes.

Richards earlier (1970) had analyzed eleven cases of parental mosaicism in which the mean age of the nine mosaic mothers was 26.9 years (similar to that of normal babies) when their Down child was born. However, the mean age of the grandmother (mother of mosaic parent) was 33.7 years, which was similar to the mean maternal age of 33.3 years of 2311 trisomy 21 cases. This suggested that the mosaic parents also arose from trisomic zygotes.

Harris, et al. (1982) summarized the findings of 26 published cases of mosaic parents (including 3 used by Richards, 1970). The 20 mosaic mothers had a mean age of 26.7 years at the birth of their first affected child, not significantly different from the mean maternal age for all births (25.8 years, $P=.24$). However, the mean maternal age of the grandmothers was 30.1 years, which was significantly elevated ($P=.004$). The mean paternal age of the six mosaic fathers was 25.2 years and that of eleven grandfathers whose ages were known was 33.5 years ($P<.01$). These elevated ages at

the time of birth of the mosaic parents suggest that some may have arisen from trisomic zygotes.

The trisomic vs. normal zygotic origin of many mosaics may be determined by studying the heteromorphisms on chromosomes #21 of the two cell lines of the mosaic and his parents. If the trisomic cell line has three different 21s, two must have come from one parent as the result of an error during meiosis I (in either the mother or the father) such that that parent gave the mosaic one copy of each of the parental #21s. If the parents have four distinguishable 21s and the trisomic cell line has two #21s that are similar and one that is different, a meiosis I error can be excluded but no definite conclusions may be drawn. Such a situation can arise either because there has been a mitotic error to produce a third #21 (similar to one in the normal cell line) or because a trisomic zygote was the result of an error in meiosis II that produced a gamete with two copies of the same parental 21. See Figure 3.

One source of bias in such studies can come from including unsuspected mosaics if such mosaics result from mitotic non-disjunction in a normal embryo. Such mosaics could be included if the normal cell line were not evident in the tissue studied. All would be designated as meiosis II errors because they would have two

Figure 3. Normal and abnormal segregation of homologous chromosomes during meiosis and mitosis.

copies of the same chromosome. The frequency of this kind of bias can not be known.

Another possible error in this kind of analysis could occur if there were crossing over in the short arm. The result would be meiosis I bivalents with dissimilar heteromorphisms. If a meiosis I error occurred after short arm crossing over, random segregation would give unlike univalents in half the cases and like univalents in half. The latter similar univalents would be mistaken for meiosis II errors. The result of short arm crossing over in analysis of a meiosis II error would be univalents with unlike heteromorphisms. All would be misinterpreted as meiosis I errors.

However, cross overs in the short arms of acrocentric chromosomes occurs only rarely. Polani (1981) summarized three studies which showed the mean range of chiasmata of #21 bivalents to be only 1.0-1.1 in women and 1.2-1.3 in men. Chandley, et al. (1976) found no chiasmata in the short arms of either D or G-group acrocentric chromosomes of five male translocation carriers. However, Mikkelsen, et al. (1980) and Magenis and Chamberlin (1981) each reported one case in which a child's heteromorphisms were different from either parent. Crossing-over in the short arm (or stalk) region is a possible explanation for these two cases,

but it probably occurs so infrequently that it is not a significant source of bias in parental origin studies.

If mosaics result from trisomic zygotes the same distribution of meiosis I and meiosis II errors should be seen in the trisomic cell line as in regular trisomy 21. If the mosaics result from mitotic misdivision of cells from a normal zygote, then the trisomic cell line will always look as if it were the result of a meiosis II error. If mosaics originate in both ways, there will be a deviation toward apparent meiosis II errors. With a large sample size, the amount of deviation would indicate the proportion from each kind of zygote.

In 1970, Juberg and Jones published the case of a child who had Down Syndrome and leukemia. Cytogenetic analysis showed that the trisomic cell line had two copies of the Christchurch chromosome, a variant #21 which has neither short arms nor satellites (Gp-). The child's normal mother had one Christchurch #21 and his father had none so he must have received both copies from the mother. deGrouchy (1970) reported a similar case in which the patient had two Gp- chromosomes and the mother had one. In both cases, the error leading to the trisomic condition probably occurred in the second meiotic division of oogenesis. Though unlikely, the

children could have been undetected mosaics in whom the normal cell line was not observed.

Beginning in 1972, more studies of the origin of the extra chromosome in trisomy 21 were published because of the advent of the fluorescent dye quinacrine which revealed inherited heteromorphisms of acrocentric chromosomes, including #21. The results of the initial group of eleven studies that were published from 1970 to 1975 are given in Table IV. There were informative results in only 26 cases, of more than 150 that had been studied.

Seventy-seven percent of the non-disjunctions had occurred in the mothers, nearly equally divided between meiosis I and II errors. Of the 23% of the errors that were paternal, five were in meiosis II and only one in meiosis I. These early studies suggested that the majority (61%) of trisomy 21 cases resulted from meiosis II errors. There is, however, serious bias in such studies. Many of these were single reports with decisions to publish based on the novelty of the idea of paternal origin. Langenbeck, et al. (1976) discussed the problem that some parental heteromorphism mating types may not allow appropriate determination of origin. For instance aa x ab can only demonstrate meiosis II errors. No mating type will allow meiosis I, but not

TABLE IV

PARENTAL ORIGIN STUDIES
DOWN SYNDROME
1970-1975

Reference	Type of Error			Proportion Informative
	Mat I	Mat II	Pat I	
Bott, 1975		3		7/59
deGrouchy, 1970		1		1/?
Emberger/Taib, 1975		1		1/?
Giraud, 1975	1	2		3/32
Hara/Sasaki, 1975 *	1	2	1	4/33
Juberg/Jones, 1970		1		1/?
Kajii/Niikawa, 1973	1			1/?
Licznerski/Lindsten, 1972	1			1/6
Mutton, 1973		1		1/?
Punnet/Kistenmacher, 1973	1		1	2/10
Robinson, 1973	4	—	—	4/12
TOTAL:	9	11	1	26/157+
	34.6%	42.3%	3.8%	38.5% Meiosis I 61.5% Meiosis II
	76.9% Maternal	23.1% Paternal	19.2%	

* One case of trisomy 13 was the result of an error at maternal meiosis I.

meiosis II, errors. Of the more than 243 families that had been studied by the publication of his paper, only 55 were informative with nearly equal distribution between meiosis I and II errors. Nevertheless, based on mating types of these families, he suggested that meiosis I errors may lead to 82% of Down Syndrome and meiosis II errors to 18%.

More recent studies have been larger and have shown more meiosis I errors than the earlier studies. Mikkelsen (1982) summarized twelve large 1975-1981 studies. Seventy-six percent of the errors were at meiosis I (63% maternal, 13% paternal); 24% were at meiosis II (17% maternal, 7% paternal). Juberg, et al. (1983) summarized all 27 studies published from 1970 to 1982. Meiosis I errors comprised 73% of the total (61% maternal, 12% paternal) and meiosis II errors 27% (18% maternal, 9% paternal). However, there is still bias toward meiosis II errors, especially in laboratories which determine both meiotic stage and parent in fewer than half the cases they study. Table V shows the results of three such studies, each with more than ten informative families. Sixty-two percent of the errors are at meiosis I and 38% at meiosis II. These proportions are significantly different from those of Table VI, which summarizes seven studies in which there

TABLE V

PARENTAL ORIGIN STUDIES
DOWN SYNDROME
At Least Ten Informative Cases
(Fewer than Half Informative)
1976-1982

Reference	Location	Source of Error			Proportion
		Mat I	Mat II	Pat I	Pat II
Mikkelsen, 1976 Hansson/Mikkelsen, 1978	Denmark	9	6	3	4
					22/72
Jacobs/Mayer, 1981	Hawaii	11	3	0	2
					16/45
Wagenbichler, 1976/1981	Germany	15	9	7	3
					<u>34/93</u>
TOTALS:		35	18	10	9
					72/210 (34%)
		48.6%		13.9%	62.5% Meiosis I
			25.0%	12.5%	37.5% Meiosis II
		73.6% Maternal		26.4% Paternal	

TABLE VI

PARENTAL ORIGIN STUDIES
DOWN SYNDROME
At Least Ten Informative Cases
(More than Half Informative)
1977-1982

Reference	Location	Source of Error			Proportion
		Mat I	Mat II	Pat I	Pat II
del Mazo, 1982	Spain	17	3	6	1
Jongbloet, 1981/2	The Netherlands	40	16	7	8
Magenis/Chamberlin, a 1977/81	Oregon	31	1	6	1
Mattei, 1979/80	France	33	8	7	3
Manning/Goodman, 1981	North Carolina	8	0	2	2
Mikkelsen, 1980 F	Denmark	28	9	3	2
Mikkelsen, 1980 Z	Denmark	21	3	7	0
Schmidt, 1981	New York	22	0	1	0
TOTALS:		200	40	39	17
					296/447 (66%)
		67.6%	13.5%	13.2%	5.7%
					80.8% Meiosis I 19.2% Meiosis II
			81.1% Maternal	18.9% Paternal	

^a Excludes possible mosaics and familial cases; includes three unpublished cases.

were more than ten cases and with results in more than half the families. Under those conditions, 81% of the errors were at meiosis I and 19% at meiosis II. These discrepancies result from the ability of laboratories with greater success to distinguish relatively subtle variations in chromosome #21; they therefore have more matings of the ab x cd type which has no meiosis II bias. Thus the 81% meiosis I error rate in Down Syndrome is the current best estimate of the actual rate.

If mosaics begin as trisomic zygotes, 81% of those that come from ab x cd matings would have three different #21 chromosomes in the trisomic cell line and random loss of one #21 to produce the normal cell line. Nineteen percent of trisomic zygote mosaics would have a meiosis II error, with two identical #21s in the trisomic cell line. There would then be a random loss of one of the #21s to produce an abb/ab pattern in two-thirds of the cases and an abb/bb pattern in one-third. The latter pattern could arise only from a trisomic zygote while abb/ab could also arise from a mitotic error in a normal ab zygote. Therefore, if mosaics arise from trisomic zygotes, the only cases in which that can not be definitely established are the 13% ($2/3 \times 19\%$) with an abb/ab pattern. All meiosis I zygotes

(abc/ab, bc, or ac) (81%) and all abb/bb mosaics (6%) can only come from trisomic zygotes. If, however, mosaics result from a mitotic error in a normal zygote or embryo, then only ab/abb patterns will be seen (100%).

The origin of the three #21 chromosomes in one phenotypically normal mosaic woman who was the mother of three Down Syndrome children has been published (Magenis and Chamberlin, 1981; Harris, et al. 1982). Ten percent of her leukocytes and 5% of her fibroblasts were trisomic. Her trisomic cell line contained two copies of one of her father's #21s and one #21 from her mother. Her normal cell line contained one of the paternal #21s and the maternal #21. Thus, she could have arisen as either a trisomic zygote as the result of a paternal error at meiosis II (6% of all free trisomy 21 is caused in this way) and lost one of the father's #21s later, mitotically, or she could have begun as a normal zygote and through mitotic mis-division produced a cell line with two copies of the paternal #21 in addition to the maternal #21. This had to occur at a time, embryologically, when a new cell line could contribute to future blood, skin (fibroblast) and germ cells. Intuitively, it seems more likely (because of her normal phenotype and low percentage of trisomic cells) to as-

sume that she began as a normal zygote and that the trisomic cells are a later minority cell population. But more cases of phenotypically normal trisomy 21 mosaicism will have to be studied to see if this presumption is general.

Additional information on the origin of trisomics and autosomal mosaics comes from cytogenetic examination of spontaneous abortions. Trisomics of all autosomes except of chromosome #1 have been seen in abortus material (Hassold, et al. 1983). Studies using chromosome heteromorphisms in the abortus and the parents to show the origin of some of these trisomics have been summarized by Meulenbroek and Geraedts (1982). Nineteen trisomy 13, 14, 15, and 22 abortuses have all been due to maternal meiosis I errors, while the eight trisomy 21 abortuses consisted of seven meiosis I errors (five maternal, two paternal) and one maternal meiosis II error. Thus all but one of 27 acrocentric trisomics have been the result of meiosis I errors. For the non-acrocentric chromosome 16, 14 were from meiosis I (12 maternal, 2 paternal) and two from meiosis II errors (1 maternal, 1 paternal).

Hassold (1982) studied the heteromorphisms of five non-acrocentric trisomic abortuses and their parents and concluded that one 46/47,+4 was the result of a meiotic

error (parent and division not determinable) while two trisomy 16 mosaics were both the result of a maternal meiosis I error. Niikawa et al (1977) reported that one mosaic trisomy 22 abortus resulted from a maternal meiosis I error. Sanchez et al (1982), studied a trisomy 9 mosaic infant whose trisomic cell line was the result of a meiosis I error.

In conclusion, it appears that while both mitotic and meiotic origin of trisomy 21 mosaicism may occur, the published case of mosaic trisomy 21 in a normal woman was possibly of mitotic origin in a normal zygote, while studies of spontaneous abortions suggest that trisomic zygotes may become mosaic by mitotic loss of an extra chromosome.

CAUSES OF NONDISJUNCTION AND MOSAICISM

Analysis of the cause of mosaic Down Syndrome requires determining whether the zygote was originally trisomic for chromosome 21, or normal. In both cases, however, there has been a mitotic mis-division in a cleavage division or later.

In normal mitosis, each chromosome aligns itself with its centromere on the metaphase plate. Each sister chromatid of the chromosome has its own kinetechore, a small, densely-staining granule of unknown composition to which the microtubules of the spindle fibers are attached. One kinetechore faces each pole. As mitosis proceeds, each kinetechore and its sister chromatid goes to opposite poles (Lewin, 1980). The segregation of chromosomes during mitosis usually occurs very accurately.

Aneuploidy could be produced from a normal cell in mitosis if the two chromatids failed to separate at anaphase. Such failure could result from improper orientation of a kinetechore so that both kinetechores faced the same direction, or from improper attachment of a kinetechore to the spindle fibers. For whatever reason, one daughter cell would have both sister chromatids of one homologue plus one chromatid from the other homologue and would be trisomic at telophase. The other

daughter cell would have no descendants because it would be haploid for that chromosome and, consequently, inviable.

The formation of a normal cell from a trisomic cell is thought to be a result of anaphase lag in which one of the sister chromatids of one of the trisomic homologues fails to be included in a daughter cell. This failure could result from poor attachment of the kinetochore to the spindle fiber. The result would be both trisomic and normal daughter cells.

Table III shows that only 0.65% of acrocentric trisomic abortuses are mosaic, compared to 7.7% of nonacrocentrics. Chandley's 1982 summary of eight surveys of trisomic spontaneous abortuses show that only 36% (338/950) involve acrocentric chromosomes, while the rest involve nonacrocentrics. These findings cast doubt on the etiological role of the nucleolar organizing regions or the satellites of acrocentrics in the production of non-disjunction in general (Miller, 1981). However, the unique properties of acrocentric chromosomes may in some way decrease the likelihood that they will be lost during mitosis to produce a normal cell line in a trisomic embryo.

If mosaic Down Syndrome cases arise from mitotic non-disjunction of trisomic zygotes or embryos as

suggested by the abortus data (Hassold, 1982), then possible causes of meiotic non-disjunction need to be considered also.

The induction of mitotic errors has not been well studied experimentally so that ideas about them are based primarily upon supposition. As with meiotic errors, many questions remain: Is there something wrong with the spindle fibers, or with the DNA or proteins of the chromosomes, or with the attachment at the kinetochore, or can all of these mechanisms be working in different cases? Ford and Roberts (1983), in a continuation of the work of Ford and Lester (1982), found that displacement from the metaphase plate was correlated with pH and calcium levels and relative rates of aneuploidy; they concluded that displacement is a facet of misdivision related to spindle dysfunction.

Frequency of Non-disjunction

It should be recognized that non-disjunction occurs frequently during meiosis in both humans and other animals. A recent summary of the cytogenetic surveys of a total of nearly 65,000 newborns (Chandley, 1982) showed that 0.31%, or one in every 300 births, was aneuploid. The rate was .13% for the autosomal aneuploids (trisomies 21, 18, and 13) and .18% for the sex chromosome aneuploids (XXY, XYY, XXX, and XO). The

aneuploid offspring observed at birth are, however, only a small proportion of the aneuploid zygotes that are present at conception. Ford (1981) proposed that 4% of all gametes are aneuploid while Chandley (1982) estimated a 10% level of aneuploidy at conception when monosomics resulting from chromosome loss during gametogenesis are included. Boue' et al (1981) estimated that 0.5% of all conceptuses have trisomy 21, but that at least 80% fail to survive until birth.

Hook (1982) estimated that there are chromosomal abnormalities in one-third of the spontaneous abortions which occur between the 5th and 27th weeks of gestation. Chandley (1982) gives a figure of 60% for chromosomal abnormalities of early spontaneous abortions and of these one-half have autosomal trisomy. Early abortions have been found with trisomy of all chromosomes except chromosome 1 (Hassold, et al. 1983). Trisomy 16 comprises about one-third of all spontaneous abortion trisomies (Chandley, 1982). Polani (1981) estimated that trisomy 16 exists in 1.2% of all conceptions. Martin, et al. (1983) analyzed 1,000 spermatozoa from 33 normal men by using in vitro fertilization of golden hamster eggs. Twenty-three (2.3%) had an extra autosome. There were three cells with an extra #21 chromosome and one with an extra #16.

Maternal Age Independent
and Dependent Causes of Non-disjunction

Because of the striking increase in the incidence of trisomy 21 with increasing maternal age, it has often been assumed that the major cause of aneuploidy is related to the long period of time between oogenic prophase (which begins prenatally in humans) and ovulation (which can occur forty or more years later). It seemed likely that something happens to the eggs during those decades which increases the probability of non-disjunction.

Penrose assumed there were both maternal age independent (class A) and maternal age dependent (class B) cases of Down Syndrome (Penrose and Smith, 1966; Smith and Berg, 1976). After eliminating patients with Robertsonian translocations (which comprise about 5% of all cases--Table I), about one-third of the cases were thought to belong to class A, the rest to class B. Below the age of 33, the majority would be class A, while above age 33 they would be class B.

They suggested that maternal age independent causes could consist approximately equally of:

- 1) Secondary non-disjunction in a mosaic or fully affected parent;

2) Genes which tended to produce non-disjunction;
and

3) Environmental disturbance of meiosis.

Secondary Non-disjunction

Trisomy 21 mosaicism in a phenotypically normal parent is one of the maternal age independent causes of Down Syndrome. Smith, et al. (1962) first reported this in the mother of two affected sons. She had trisomic cells in both her blood and fibroblasts. Jagiello (1981) summarized information on 36 mosaic parents: 24 mothers and 12 fathers. Six of the mothers, but only one of the fathers, had two trisomy 21 children. Harris, et al. (1982) give information on an additional two mosaic mothers with one trisomy child and three mosaic mothers, each with three trisomy 21 children. Combining these two summaries, there are 29 reported cases of mosaic mothers (18 with one affected child, 8 with two, and 3 with three). Among twelve mosaic fathers only one had more than one affected child.

In many of these cases only the mother was karyotyped. It is not clear how much of the increased incidence of maternal compared to paternal mosaicism can be ascribed to this biased testing. If phenotypically normal mosaics who have trisomy 21 children arise from mitotic errors, as may be the case in Harris, et al.

(1982), there is no reason to expect the nondisjunction to occur more frequently in women than in men. Because mitosis continues after birth in the germ cells of men, but not of women, it would seem possible that men might be more likely to have numerical aneuploid gametes as a result of mitotic errors.

One possible explanation may be that mosaic men, like fully-affected trisomy 21 men, may tend toward infertility. Johannisson, et al. (1983) reported a study of meiosis in an 18-year-old Down male. There was nearly complete spermatogenic arrest with few secondary spermatocytes, rare spermatids and no mature sperm. The extra #21 chromosome was seen at metaphase I as a univalent or, rarely, as a trivalent. All but one of ten other studies of trisomy 21 males also reported some degree of spermatogenic arrest.

Fertility is not absent in trisomy 21 women, however. Smith and Berg (1976) report 23 fully affected Down women who have had 24 children. All but five of the women had been karyotyped to show that they were 47,XX,+21. The children included nine with Down Syndrome, two other retarded children, three stillborns (including a set of male twins) and ten normal children. Thirty-nine percent of the pregnancies had resulted in trisomy 21 children. This figure is less than the 50%

expected from production of equal numbers of eggs with 23 and 24 chromosomes, but more than would be expected considering that about 80% of Down conceptions are probably aborted. However, the reported cases are probably not an unbiased sample of pregnancies of Down women.

Two oocytes have been described in women with trisomy 21. Jagiello (1981) reported 22 bivalents and one trivalent at metaphase I in a trisomy 21 mosaic, while Polani, et al. (1982b) described a metaphase I oocyte with 23 bivalents and one univalent.

On the basis of dermatoglyphic studies which showed some parents of Down Syndrome offspring to have patterns more similar to Down patients than to normals, Penrose (1967) estimated that 10% of mothers and 1% of fathers of Downs are mosaics.

Priest, et al. (1973) compared the Walker dermal index scores of 296 parents of Down Syndrome children and 204 controls. They estimated that 11% of mothers and 8% of fathers could be mosaics.

Rodewald, et al. (1981) used their general dermatoglyphic index of Down Syndrome (adding bilateral symmetry of the patterns to the Walker index) to compare 150 Down patients to 550 controls. There was a region in which 4% of the patients overlapped with 3.1% of the

controls. Of 17 mosaic Down patients, 15 were in the Down region, one in the overlap region, and one in the normal region. They discovered among 146 parents of Down children that 4% were in the Down region based on their index values, and 16% in the overlap region (compared to 3.1% of controls). They mention that because dermal ridge differentiation occurs from the second to seventh prenatal month, dermatoglyphic abnormalities will remain even if selective forces have led to the loss of a trisomic line in the lymphocytes.

Loesch (1981) compared the distribution of individual dermatoglyphic discriminant scores among parents of Down children and parents of non-Down children. Values strongly indicative of mosaicism were found in 9.5% of Down parents and 1.7% in control parents. She postulated that 7% of both mothers and fathers in her sample would have trisomy 21 mosaicism.

Schmidt, et al. (1981) compared the Hopkins index in 131 mothers and 95 fathers of Down children to 200 controls. Twelve percent of the mothers and 5% of the fathers had either a positive or overlap index compared to 2% of the control females and 8% for the control males. Parental origin studies in 40 families indicated that all cases were the result of meiosis I errors (22 maternal, 1 paternal, 17 unknown). Of the 16 mothers

who were 35 or younger, three had overlap scores, while no mother older than 35 had such a score. They considered that gonadal mosaicism may have been present in those three mothers although studies of 100 cells each of both blood and fibroblasts from them showed no trisomic cells.

These high estimated rates of mosaicism among parents (5-10% of mothers) have not been confirmed in large studies of Down Syndrome families.

Mikkelsen (1970) found one mosaic mother among 84 women who were less than 30 years old when they had their first affected child. She summarized four other studies in which one mosaic mother was found among 292 parents examined (117 mothers, 64 fathers, 111 sex unspecified). Stevenson et al (1969) reported that among 100 couples with a Down Syndrome child one mother was a mosaic. Fifty-four additional mothers were tested and none was found to be mosaic.

While the studies through 1970 suggested that about 1% of mothers of Down Syndrome children are themselves trisomy 21 mosaics, the large parental origin studies from 1975 to 1982 do not confirm this.

Table VII shows that of 538 couples who are parents of one Down Syndrome child and who have been studied cytogenetically in order to establish the origin of the

TABLE VII
PARENTAL ORIGIN STUDIES
ONE DOWN CHILD
NO MOSAIC PARENTS

Reference	#Couples	#Mosaics	Minimum #Cells Counted	Minimum % Mos. Excluded 95% Confidence ^a
del Mazo, 1982	48	0		
Jacobs/Mayer, 1981	45	0		
Manning/Goodman, 1981	15	0	20	14%
Mattei/Ayme, 1980	70	0	50	6%
Mikkelsen, 1980	110	0	10	26%
Mikkelsen, 1976	40	0	10	26%
Roberts/Callow, 1980	20	0	10	26%
Jongbloet, 1981	100	0		
Schmidt, 1981	40	0	100	
Magenis, 1981	<u>50</u>	<u>0</u>	<u>16</u>	<u>18%</u>
	538			

^a Hook (1977)

child's three #21 chromosomes, none have been found to be mosaic. In addition, Mehes (1978) studied 60-100 mitoses of 25 mothers and 19 fathers of Down children and found no mosaics among the 44 parents. If parental mosaicism is more than a very rare cause of Down Syndrome, studying lymphocytes during the parents' child-bearing years is not an efficient way to discover the mosaicism.

Genetic Causes of Non-disjunction

The genetic control of meiosis has been somewhat elucidated by the study of meiotic mutants. Golubovskaya (1979) described mutants which affect:

- 1) meiotic patterns (entry into meiosis or substitution of mitosis for meiosis--observed in maize);

- 2) synapsis (asynapsis - only univalents are present in the metaphase I plate - or abnormal synaptonemal complex formation--wheat, maize, *Drosophila*);

- 3) desynapsis (homologue co-orientation disrupted after pairing--various plants);

- 4) cross-over frequency (*Drosophila*);

- 5) chromosome disjunction (spindle apparatus failures or precocious centromere division--*Drosophila*)

- 6) single chromosome behavior (*Drosophila*);

and

7) impairment or loss of second meiotic division (plants).

There is little evidence for the existence in humans of genes that predispose to non-disjunction. Baker (1976) noted four possible kinds of evidence for meiotic mutants in humans:

1) clustering of chromosomal abnormalities within families,

2) heritable differences of morphology of specific mitotic chromosomes,

3) genetic syndromes which increase mitotic chromosome instability and defective DNA repair processes;

and

4) abnormal meiotic chromosome behavior in some sterile men.

There are anecdotal reports of sibships with multiple kinds of aneuploids (Hecht, 1977) but no systematic studies have shown that there are more of these families than would be expected from chance occurrence of rare events. There are no currently known meiotic consequences of "coiler" or fragile chromosomes or of the repair-defective chromosome instability syndromes. Templado, et al. (1981) summarized the meiotic anomalies which have been described in subfertile men. The most

common anomaly involves a decreased number of chiasmata and later desynapsis of bivalents. There are often pairing and synaptonemal complex anomalies. The desynapsis may be partial or complete and may affect all or a few bivalents in all or a few cells. More rarely asynapsis occurs, with the abnormal pairing evident from the beginning of prophase I. Because of their subfertility, these men are not described as having aneuploid offspring. Therefore it is not known if their abnormal meiotic synapsis is related to the nondisjunction that occurs in normal males.

Alfi, et al. (1980) reported that closely related parents in Kuwait were more likely to have Down children ($n=20$) than nonrelated parents ($P<.005$). They suggested that the children could be homozygous for a gene that results in mitotic non-disjunction with loss of the monosomic cell to produce a complete or mosaic trisomic. Alternatively, the parents in this frequently consanguineous population could have been homozygous for a recessive gene which leads to meiotic non-disjunction. Yokoyama, et al. (1981) analyzed mathematically the proportion of trisomies that would come from consanguineous marriages if single or multiple recessive genes induce non-disjunction. The proportion of trisomies would increase with increasing consanguinity for mitotic

non-disjunction but would decrease for meiotic non-disjunction. In conclusion, they state that no published data support the hypothesis that recessive genes induce non-disjunction in humans, but that testing the hypothesis requires only knowing the magnitude of parental consanguinity and whether mitotic or meiotic non-disjunction has occurred.

Stallard, et al. (1981a; 1981b) compared the frequency of hypermodal cells in 36 parents of 19 aneuploid offspring (1/124) and found a significant increase compared to age-matched controls (1/886) ($P < .01$). Staessen, et al. (1983) compared the number of hyperploid cells in 8 couples who had trisomy 21 children (1.4%) to 8 control couples who had at least two normal children (0.3%) ($P < .02$). They suggest that mitotic non-disjunction may express a condition that could also lead to non-disjunction during meiosis, such as abnormal spindle assembly or increased chromosome association.

Environmental Causes of Non-disjunction

Possible environmental causes of non-disjunction in experimental animals have considered both clastogens and spindle poisons. Russell (1979) discussed the importance of considering both sex and meiotic stage when testing for induction of non-disjunction. If reduced crossing over or synapsis contributed to it, the sensi-

tive stage would be before or during crossing over, while kinetechore damage could occur at any stage and spindle damage would occur between prophase and metaphase.

Sugawara and Mikamo (1980) induced meiotic non-disjunction and anaphase lagging in Chinese hamster primary oocytes by using the mitotic spindle inhibitor colchicine, which inhibits tubulin polymerization. Chromosome analysis of over 2,000 oocytes showed an increase in aneuploidy from 2% in the control group to 26% in the experimental group. Tates (1979) found a significant ($P=.016$) increase in sex chromosome non-disjunction in the field vole after treatment with the spindle inhibitors vincristine, a cytostatic drug, and MBC, a benzimidazole pesticide. Ramel and Magnusson (1979) induced sex chromosome non-disjunction in male and female *Drosophila* by the use of colchicine, organic mercury, lead, and tin compounds, all known spindle inhibitors.

There have been many studies of the induction of aneuploidy by X-irradiation (reviewed by Hansmann and Probeck, 1979). Radiation-induced chromosome non-disjunction has been described in female mice by Tease (1982a; 1982b) and in male mice by Russo, et al. (1983). Russo, et al. and Tease all mention that there is an

increase in the number of chromosome fragments in cells that have induced aneuploidy, suggesting that direct damage to chromosome structure may be responsible for non-disjunction as well as chromosome breakage. Cyclophosphamide, an antineoplastic drug, also increased the frequency of both hyperhaploid cells and those with chromosome breaks in mouse secondary spermatocytes (Pacchierotti, et al. 1983).

Some epidemiological studies have suggested that there may be an increased incidence of Down offspring born to mothers who have been exposed to x-irradiation (Uchida, 1979, 1981), but positive results have not been found in all studies (Sankaranarayanan, 1979).

Paternal Age Effect

A paternal age effect has not been proven to be a contributing factor in the etiology of trisomy 21. Because of the usual correlation of maternal and paternal ages it is difficult to separate out a specifically paternal effect. Stene, et al. (1977) analyzed the parental ages of 224 Danish Down subjects and concluded there was an increasing incidence for a given maternal age with advancing paternal age. There was a significant increased risk for men over 55 years of age. Erickson (1978) analyzed the parental ages of over 4000 Down infants and found no independent paternal age ef-

fect. However, Stene and Stene (1978) criticized Erickson's statistical test and stated that there were too few older fathers in his sample to permit any conclusions. Hook (1980, 1981) concluded that the then available literature suggested a doubling of risk for men over 55, but no effect for younger men. Stene, et al. (1981a) analyzed parental ages of 65 cases of trisomy 21 that were found among amniocenteses of women who were 35 or older. They concluded that a strong paternal age effect existed for fathers over 40 years of age. Roth, et al. (1983) examined parental ages of over 600 French Down children and found no evidence for a significant paternal age effect. They concluded that there may be a weak paternal age effect but that it isn't always observed because of population fluctuations and methodological artifacts.

Maternal Age Effect

Age dependent causes of non-disjunction (Penrose and Smith, 1966; Smith and Berg, 1976) would be related to deterioration of the meiotic mechanism in oocytes, such as breakdown of the synaptonemal complex, failure of the nucleolus to break down, or degeneration of spindle fibers. In any case, it seemed likely that the great increase in incidence with age was related to the long period of time the oocytes spent in dictyotene

before the first meiotic division is completed before ovulation. This would suggest that most Downs born to older mothers would be the result of maternal meiosis I errors. This hypothesis can be tested by comparing parental origin study results from both young (twenty-five or younger) and old (thirty-nine or older) mothers. All studies that have mothers in both categories may be included, even those with a bias toward meiosis II errors because the bias will hold for both age groups.

Table VIII shows that only 61% of all Downs born to women who are forty or older come from meiosis I errors and 79% of all the maternal errors are at meiosis I.

The results for mothers who are 25 or younger are nearly the same: 59% of all errors are at maternal meiosis I and 77% of all maternal errors are at meiosis I. Therefore, the age dependent increase in Downs born to older mothers is not from an increase in meiosis I errors in aging oocytes. Whatever causes non-disjunction in young and old mothers is operating in the same way, but more frequently in older mothers. Because of the lack of differences in origin among younger and older mothers, the role of maternal age in Down Syndrome has been questioned and the suggestion made that the maternal age effect may reflect a decreased rate of spontaneous abortions of trisomy 21 fetuses in older

TABLE VIII

PARENTAL ORIGIN STUDIES WITH AT LEAST
ONE MOTHER OLDER THAN THIRTY-NINE AND
ONE TWENTY-FIVE OR YOUNGER

Thirty-Nine or Older

Study	Mat I	Mat II	Pat I	Pat II
del Mazo, 1982	4	2	4	
Jacobs/Mayer, 1981	1	1		
Magenis, 1981	5		1	1
Mattei, 1980	3		1	1
Hansson/Mikkelsen, 1978	1			
Mikkelsen, 1980	8	2		
Wagenbichler, 1976	<u>5</u>	<u>2</u>	<u>2</u>	<u>1</u>
	27	7	8	2
	61%		18%	
80% MEIOSIS I		16%		5%
20% MEIOSIS II				
	77% Maternal		23% Paternal	

TOTAL: 44

Maternal errors: 79% Meiosis I

Twenty-Five or Younger

Study	Mat I	Mat II	Pat I	Pat II
del Mazo, 1982	1			1
Jacobs/Mayer, 1981	5	2		1
Magenis, 1981	9		3	
Mattei, 1980	10	2	2	
Hansson/Mikkelsen, 1978	1	3	2	1
Mikkelsen, 1980	14	4	4	1
Wagenbichler, 1976	<u>3</u>	<u>2</u>	<u>1</u>	<u>1</u>
	43	13	12	5
	59%		16%	
75% MEIOSIS I		18%		7%
25% MEIOSIS II				
	77% Maternal		23% Paternal	

TOTAL: 73

Maternal errors: 77% Meiosis I

mothers rather than differences in the induction of aneuploidy [Mattei, et al. (1980); Sved and Sandler (1980); Ayme and Lippman-Hand (1982)].

Carothers (1983) and Warburton, et al. (1983) have both disputed Ayme and Lippman-Hand's analysis and they (Ayme and Lipman-Hand 1983) in turn have responded in defense of their interpretation. The issue is unlikely to be settled until larger numbers of trisomy 21 spontaneous abortion cases which include maternal age are published.

The maternal factor or factors that lead to increasing non-disjunction with increasing age is not known. The strength of maternal age effect as a contributing factor to trisomic spontaneous abortions varies among the different chromosomes. It is strongest ($P=.001$) for both acrocentric (#15, #22) and nonacrocentric (#18, #20) small chromosomes (based on 362 trisomic abortions, Hassold, et al., 1980a). There was also a substantial ($P=.01$) increased maternal age effect in trisomy 21 and a smaller effect ($P=.05$) in trisomy 16. No significant maternal age effect was shown for chromosomes 13, 14, and 17 because of the small number of trisomic subjects. The maternal age effect for the large chromosomes #1 through #12 is small. They suggest that age-dependent loss of chiasmata may lead to the

formation of univalents from small bivalents while the larger chromosomes have more chiasmata and are more likely to be held together even if some chiasmata are lost.

Two major effects of chiasmata on non-disjunction have been proposed (Polani, 1981; 1982a). One assumes that normal pairing has occurred and chiasmata have formed but that premature desynapsis occurs during the long female dictyotene stage as a result of environmental, hormonal, or other aging factors. The other hypothesis, proposed by Henderson and Edwards (1968) is that there is a production line of oocytes that causes differences in early and late formed oocytes. Those formed early in fetal life have more chiasma and are ovulated first. The later-formed oocytes, which have fewer chiasma, are ovulated later in life and are more prone to non-disjunction. Speed and Chandley (1983) have been unable to find evidence of differences in the oocytes produced during fetal days 15 to 19 in female mice. Univalents were no more common later in gestation. There was occasional desynapsis of bivalent 19 at days 18 and 19 but because trisomy 19 is not over-represented in mid-term mouse embryos the premature separation is apparently not correlated with the production of trisomic zygotes. The lack of correlation

between formation of univalents and meiotic non-disjunction was also described by Sugawara and Mikamo (1983) in young and old Chinese hamsters. Aged hamsters had a significantly higher incidence of both univalents at meiosis I (9% vs. 2%, $P < .05$) and aneuploidy in meiosis II oocytes (3.6% vs. 1.5%, $P < .05$). However, all but one of 23 univalents in older mice were found among the three small D-group chromosomes, but non-disjunction occurred in all four chromosome groups. Therefore, non-disjunction occurs independently of univalent formation.

There are a variety of explanations for non-disjunction that involve female hormone levels. Crowley, et al. (1979) suggested that older women may have a longer interval between the resumption of meiosis and ovulation. This time interval would allow terminalization of chiasmata and the production of univalents. However, the idea that chiasmata actually terminalize has been questioned by Imai and Moriwaki (1982). After studying meiosis in male mice they believe that terminal associations of bivalents do not represent terminalized chiasmata.

Lejeune and Prieur (1979) compared retrospectively the use of oral contraceptives among 730 mothers of trisomy 21 children and 1035 mothers of abnormal children who did not have chromosomal aberrations.

Among mothers 30-38 years old, there was a significant excess of trisomy 21 children among those who had taken the pill for more than one year and those who had conceived less than six months after cessation of pill taking. Janerich, et al. (1980), however, found no association with oral contraceptive use in 103 mothers of Down offspring compared to 612 mothers of children with other kinds of malformations and 715 matched controls.

The estrogenic compound, clomiphene, which has been used to induce ovulation, has also been implicated in the conception of aneuploid offspring (Simpson, et al., 1982).

Jongbloet, et al. (1982) studied the month of birth of 287 cases of Down Syndrome in whom the parent and meiotic stage of origin were known. Maternal meiosis I errors occurred more frequently during seasonal transitions of increasing or decreasing ovulation rate as predicted by the hypothesis of seasonal pre-ovulatory over-ripeness ovopathy. The transitional periods cause a delay in ovulation.

CHAPTER II

MATERIALS AND METHODS

TECHNIQUES

Blood Culture

Peripheral venous blood is drawn into a sterile syringe which contains the anticoagulant, heparin, and cultured by either the plasma-lymphocyte or whole blood method. The cultures are incubated for 66-72 hours at 37° C.

Plasma-lymphocyte method. The blood is gently centrifuged or allowed to settle for a few hours until the red cells settle to the bottom of the tube. The clear plasma at the top of the tube and the white cell containing buffy coat between the red cells and plasma are drawn into a sterile tube. One ml of the plasma buffy coat mixture is added to each of two to four 30 ml culture flasks which contain 10 ml of RPMI 1640 media (9 ml 1640, 1 ml fetal calf serum, .05 ml of the antibiotic gentamicin) and 0.1 ml of the mitogen phytohemagglutinin (PHA).

Whole-blood method. Eight to thirteen drops of whole blood are added to 5 ml of RPMI 1640 media (as

above) in 15 ml centrifuge tubes.

Harvest and Slide Making

One to one-and-a-half hours before the cells are harvested, one to two drops of the mitotic inhibitor colcemid is added to each flask or tube. At the end of the colcemid incubation time, the material in flasks is poured into 15 ml tubes and the tubes are centrifuged at 1000 rpm for 6-7 minutes. Then the supernatant is aspirated, the button of material at the bottom is gently resuspended, and 3 ml of the hypotonic KCL (0.075 M) is slowly added. The tubes are recentrifuged, the supernatant drawn off and another 3 ml of KCL added. After another centrifugation and supernatant removal 3 ml of 3:1 methanol: acetic acid fix is added. After 20 minutes or more, the cells are spun, aspirated and more fix is added.

Slides are made by dropping fix-diluted cell suspensions onto wet slides.

Fibroblast Preparation

All fibroblasts were cultured by the Clinical Cytogenetics Laboratory, Oregon Health Sciences University.

Chromosome Stains

Q-bands. Slides are stained for 10-15 minutes in a solution of 3 mg quinacrine mustard dihydrochloride,

10 ml distilled water, and 30 ml pH 7.0 MacIlvaine's buffer (Caspersson, et al. 1971). The slides are mounted in a sucrose syrup (6 grams sucrose in 10 ml pH 7.0 buffer) with a clean cover slip.

The mechanism of Q-banding is not known. Quinacrine apparently binds along the entire length of the chromosome, but differential quenching of the fluorescence in some areas produces the positive bands in A-T rich, late-replicating regions of the chromosomes (Sumner, 1981).

R-bands. Slides are stained sequentially in the dark with 7-amino Actinomycin D (0.7 microg/ml pH 7.0 buffer) for 3 minutes, chromomycin A₃ (0.5 mg/ml) for 20 minutes, and Distamycin A (0.1 mg/ml) for 5 minutes (Schweizer, 1980). A clean coverslip is mounted with glycerol.

The mechanism of the R-banding is also unknown. Actinomycin-D and chromomycin A₃ are both G-C specific fluorescent dyes (Latt, et al. 1980), while the antibiotic distamycin A acts as a non-fluorescent A-T specific counterstain (Schweizer, 1980).

Photomicroscopy

Slides are examined with a Zeiss Photoscope III with a KP 490 excitor, FT 510 reflector, and LP 520 nm barrier filter package. Cells with good chromosome

morphology are photographed with Kodak technical pan film 2415. The chromosomes 21 or 13 are printed at different exposure times to illustrate brightness differences of short arm and satellite regions.

PATIENTS

Eighteen of the twenty patients involved in this study were identified as possible mosaics by the Clinical Cytogenetics Laboratory of the Oregon Health Sciences University. One was first studied in Idaho and one in Eugene, Oregon.

Frozen chromosome suspensions were available from five patients. Fresh slides were made and metaphase cells were examined microscopically for the presence of both trisomy 21 or 13 and normal cell lines. Mosaicism was uncertain in three patients who had fewer than 12% normal cells and was confirmed in two samples, but none of these patients was available for further study (Table IX). The quality of the material and the heteromorphisms in the two definite mosaics was too poor to permit any inferences about the origin of the two cell lines. New studies were made of three patients who were eliminated from the study because they had fewer than 5% normal cells (Table X).

After eliminating those patients in whom mosaicism was not certain and those who were unavailable, twelve

TABLE IX

CONTINUATION OF ORIGINAL STUDIES
PATIENT UNAVAILABLE

A. Mosaicism Uncertain

Study (Mo/Yr)	Birth Mo/Yr	Sex	Mat Age	Pat Age	Orig. Study	Trisomic Cells Extension	Total
GL 5674 (3/76)	9/51	M			14/18	86/87 (ML519)	95%
GL 6771 (5/77)	5/77	M	23	24	8/9	16/18 (ML1088)	89%
GL 7647 (3/78)	3/78	M	36	50	13/15	25/27 (ML1293)	90%

B. Mosaicism Confirmed

GL 5576 (1/76)	1/76	M	34	37	17/23	48/60 (ML495)	78%
GL 6640 (4/77)	6/76	M			6/10	46/86 (ML985)	54%

TABLE X

STUDIES OF NEW BLOOD SAMPLES
MOSAICISM NOT CONFIRMED

Original Study (Mo/Yr)	Birth Mo/Yr	Sex	Mat Age	Pat Age	Orig. Study	Trisomic Cells New Study	Total
GL 305 (3/67)	7/66	F	18	21	30/31	(11/81)	96%
GL 395 (6/67)					61/67		
GL 868 (5/68)	2/68	F	26	22	4/6	(9/80)	96%
Unknown	6/65	F				(11/81)	97%

patients were suitable for further study. Frozen suspension from the newborn period was available for one trisomy 21 mosaic (Case 1) and one trisomy 13 mosaic (Case 9) and from both their parents. Fresh blood from three typical trisomy 21 mosaics (Cases 2, 4, 6), one infant with leukemia but no other signs of Down Syndrome (Case 5), and one trisomy 13 mosaic (Case 10) and all their parents except one father were cultured. Blood from the patient only was obtained from one adopted Turner/Down mosaic (45,X/47,XX,+21) (Case 7) and one institutionalized Down adult (Case 3). Fresh blood was obtained from two patients who had been involved in an earlier study of parental origin of Down Syndrome (Cases 8 and M-11) and frozen suspension studied from a third patient involved in that study (Case M-6). (Case numbers M-6 and M-11 refer to Cases 6 and 11 in Magenis 1977; 1981.) Frozen suspension of fibroblast tissue culture from four patients was also available for further analysis (Cases 4, 5, 7, 9).

In addition to the patients who were suspected of having Down Syndrome because of their phenotype, two families who had more than one Down Syndrome child of the 47,+21 type were examined because of the possibility of parental mosaicism. Family A had two trisomy 21 children and six normal children. Family B had a set of

boy-girl trisomy 21 twins after having two normal children, and a boy, said to have Down Syndrome, who died in Mexico at age two and who had never been examined cytogenetically. Both blood and fibroblasts were examined in both parents in Family B.

Well spread metaphases were examined and scored for the presence of trisomy 21 or 13. Pictures were taken of cells that had good morphology of the heteromorphic regions of the #21 or #13 chromosomes. Cells with two #21s or #13s were photographed only if there were no uninterpretable chromosome overlaps. Serial printing (Overton, et al 1976), a technique in which different exposure times are used in printing the same chromosomes, was used to enhance differences of brightness of satellite or short arm regions. Some chromosomes with very pale satellites need to be printed with very short exposure times to make the satellites visible, while brighter satellites can be distinguished with long exposure times which can reveal the brightness of the satellite relative to that of the long arm. The chromosomes of the trisomic cell line were labelled "1", "2", and "3" and those of the normal line "1" and "2". The parents' chromosomes were labelled "A" and "B" in one parent and "C" and "D" in the other.

All patients and parents were initially studied

using the fluorescent stain quinacrine (Q-banding). In one case of mosaic trisomy 21 (Case 5) and one case of familial trisomy 21 (Family B) R-banding was performed because Q-banding did not reveal informative heteromorphisms. The R-banded chromosomes were numbered 1, 2, 3, and 4 based on the brightness of the stalk region.

The author and two others who were experienced in interpreting fluorescent heteromorphisms examined the patient's and parents' chromosomes to establish the origin of the patient's trisomic and normal chromosomes. Results reflect agreement among all three.

CHAPTER III

RESULTS

Table XI shows the proportion of trisomic cells (± 2 standard errors) for the twelve mosaic patients included in this study. The six known trisomy 21 mosaics who were studied more than once have all shown a consistent decrease in the proportion of trisomic cells in their lymphocytes (Cases 2-7). One trisomy 13 mosaic showed a slight increase in trisomic cells (Case 10). The three Downs who were included in the earlier parental origin study all now have 94% (Case 8) or 98% (Cases M-6 and M-11) trisomic blood cells. In Case 8, it is possible that normal cells do not always have the same two #21 chromosomes so that a high degree of random loss of #21s may be occurring.

The original, zygotic cell line could be established in six of the seven certain trisomy 21 mosaics (Table XII). Four arose from trisomic zygotes. Three of the cases from trisomic zygotes are illustrated in Figure 4. Each chromosome is shown at two different exposure times to demonstrate differences in satellite brightness. At the brightest (top) exposure, all the chromosomes except 1-A and 3-A have a distinct satellite

TABLE XI
PROPORTION TRISOMIC CELLS
MOSAIC PATIENTS

Case No.	Birth Mo/Yr	Sex	Age at Study	# Cells Examined	Trisomic Cells $\pm$ 2 S.E.
1	11/80	M	nb	243	.87 $\pm$.04
2	9/82	F	nb 6 wk	167 270	.87 $\pm$.06 .84 $\pm$.04
3	7/62	F	5 yr 15 yr 20 yr	14 152 200	.21 $\pm$.22 .10 $\pm$.05 .07 $\pm$.04
4	12/79	M	nb 1 mo 10 mo	9 40 94	.67 $\pm$.32 .92 $\pm$.08 (skin) .23 $\pm$.08
5	1/83	M	nb nb 2 mo 4 mo	168 21 40 320	.28 $\pm$.07 .90 $\pm$.13 (bm) ^a .00 (skin) .00
6	1/79	M	nb 3 yr	25 537	.56 $\pm$.20 .13 $\pm$.03
7 ^b	12/70	F	nb nb 6 yr 12 yr	180 90 74 160	.52 $\pm$.07 1.00 (skin) .50 $\pm$.12 .36 $\pm$.08
8	2/58	F	14 yr 24 yr	31 269	.90 $\pm$.11 .94 $\pm$.03
9 ^c	3/82	F	nb nb	248 21	.33 $\pm$.20 .15 $\pm$.04 (skin)
10 ^c	8/80	F	nb 1.5 yr	19 63	.37 $\pm$.22 .48 $\pm$.12
M-6	7/74	F	nb 10 mo	14 81	.43 $\pm$.26 .98 $\pm$.03
M-11	1/56	M	18 yr 26 yr	13 89	.62 $\pm$.27 .98 $\pm$.03

^a bone marrow

^b 45,X/47,XX+21

^c 46,XX/47,XX+13

TABLE XII

RESULTS OF ORIGIN STUDIES
46/47, +21 OR 13 MOSAICS

Case No.	Mat Age	Pat Age	Heteromorphisms		Mat	Origin of Trisomic Cell Line
			Trisomic/Normal	Pat		
TRISOMY 21						
<u>Meiotic Errors</u>						
1	39	34	ABC/AB	AA	BC	Mat I
2	21		ACD/CD		CD	Mat or Pat I
3	26	43	ABB/BB or ABC/BC			Mat or Pat I or II
6	26	27	ACD/AC	AB	CD	Mat I
<u>Mitotic or Meiotic II Errors</u>						
4	29	29	ACC/AC	AB	CD	Mat II or Mitotic
5	20	22	Q: ACC/AC R: 2,4,4/2,4	AB 2,3	CC 4,1	Mat II or Mitotic
<u>Uninformative</u>						
7 ^a	24		ABB/AB or ABC/AB			
<u>Previous Parental Origin Study</u>						
M-6	22	23	ABC/AB, BC	AB	CD	Pat I
M-11	25	27	AAC/AC	AB	CD	Pat II or Mitotic
8	22	37	ACD/	AB	CD	Mat I
TRISOMY 13						
<u>Uninformative</u>						
9	39	38	ACC/AC	AB	CC	Mat or Mitotic
10	39	43	ABB/AB	AB	AB	

^a 45,X/47,XX+21

Figure 4. Q-banded chromosomes of the trisomic and normal cell lines of three mosaic trisomy 21 cases in which the trisomic line resulted from a meiotic error. Each chromosome is printed at two different exposure times to reveal differences in brightness of the satellites.

Cases 1 and 2 each have three different #21s in their trisomic cell lines. The #21s vary in both satellite brightness and stalk length.

Case 3 has one #21, "A", which is in her trisomic, but not her normal, cell line.

which is separated from the rest of the chromosome by a non-staining stalk region. At the dimmer (bottom) exposure, the pale satellites are no longer visible in chromosomes 1-C and 2-D, but are still visible in the brighter 1-B, 2-A, 2-C, and 3-B.

The trisomic origin of the mosaics could be established in three cases because the trisomic cell line had three different heteromorphic chromosomes. In the two cases in which both parents had also been studied, the errors were in maternal meiosis I (Cases 1 and 6). In the third meiosis I case, the father was not available. The chromosome heteromorphisms are consistent with a maternal meiosis I error, but a paternal error also could have occurred (Case 2).

The origin of the fourth trisomic case was revealed because there was a chromosome in the trisomic cell line that was not present in the normal cell line (Case 3). The trisomic cells had one #21 with a pale, indistinct satellite (A) and two #21's with bright satellites (B). Only the two bright #21's were in the normal cell line. Because her parents are not available for study it isn't known if the "B" chromosomes of the normal line are two copies of one parental chromosome or separate copies of a similar chromosome from each parent. Therefore the trisomic cell line could be the result of either a

meiosis I or meiosis II error.

In two cases the trisomic line came from either a meiosis II error or from a mitotic error in a normal individual (Cases 4 and 5). In both cases there were two copies of one of the mother's #21 chromosomes in the trisomic cell line (Figure 5).

In Case 4, the child's trisomic cell line had two copies of the mother's chromosome "C" which had a distinct, but pale, satellite. In Case 5, the mother's two #21 chromosomes were very similar with quinacrine banding. The child obviously had two maternal #21s in his trisomic cell line, but it could not be determined if they were one copy of each of her two #21s or two copies of one of them. By R-banding, however, her #21s were very different. One had a very pale stalk region while one was extremely bright ("4"). The child had two of the very bright #21s, or two copies of a single maternal #21.

In both cases of trisomy 13 mosaicism, the parents had one or two similar chromosomes so that it was not possible to determine origin precisely. In Case 9, both of the mother's #13 chromosomes had bright centromeres and pale satellites and it was agreed that the child's trisomic cell line contained two of them but they were too similar to reach agreement on whether the child had

Figure 5. Chromosomes 21 of the parents and trisomic and normal cell lines of two mosaic trisomy 21 cases.

Case 4 (Q-banding): The child's trisomic cell line has two copies of his mother's chromosome "C" which has a distinct, but pale, satellite and a non-staining stalk.

Case 5 (R-banding): The child's trisomic cell line has two copies of his mother's extremely bright chromosome "4".

two copies of one or one copy of each. In Case 10, none of the four parental #13s had distinctive heteromorphisms.

The parents of the Turner/Down mosaic (45,X/47,XX,+21) are not available for study (Case 7). She has two very similar #21's, each with a stalk and a very pale satellite and with positive silver staining, in her trisomic cell line and one of them in her Turner cell line.

Of the three cases that were part of an earlier parental origin study (Magenis and Chamberlin, 1981), and in whom mosaicism cannot be proven, two are the result of meiosis I errors (Case M-6:Pat I; Case 8:Mat 1) and one is the result of either a paternal meiosis II or a mitotic error (Case M-11).

Results were obtained in the two families which each had two trisomy 21 children available for study (Table XIII). All four Down children were the result of an additional chromosome from the mother. In both cases, one child was the result of an apparent maternal meiosis I error and one child resulted from a maternal meiosis II error. In Family B, three of the four parental #21 chromosomes were similar with Q-banding. However, with R-banding all four had different degrees of brightness of the stalk so that parental origin could

TABLE XIII

RESULTS OF ORIGIN STUDIES
TRISOMY 21 SIBSHIPS

Family	Child	Birth Mo/Yr	Sex	Mat		Pat		Down		Heteromorphisms		Origin
				Age	Age	Age	Age	Mat	Pat	Mat	Pat	
A	1	1/58	M	29	31			ACC	AB	CD		Mat II
	2	10/59	F	31	33			ACD	AB	CD		Mat I
B	1	12/82	M	23	27			Q: ABB R: 1,4,4	AB 1,3	BB 2,4		Mat II
	2	12/82	F	23	27			Q: BBB R: 3,2,4	AB 1,3	BB 2,4		Mat I

be established in both children. Blood from all parents and fibroblasts from the mother (300 cells) and father in Family B showed no evidence of mosaicism.

CHAPTER IV

DISCUSSION

The results of this study show that trisomy 21 mosaic cases can arise by loss of a #21 chromosome from a trisomic embryo. Four of the nine cases investigated for this study could only have come from trisomic zygotes, while two were the result of either meiosis II errors in the mother or mitotic errors in a normal embryo. These results are combined in Table XIV with those of four other studies of autosomal mosaics for a total of nine cases of trisomic zygotes (meiotic errors) and three cases of either a meiosis II or mitotic error. Of the nine meiotic errors, five occurred during maternal meiosis I, two during either maternal or paternal meiosis I, and two during either maternal or paternal meiosis I or II. These results show that of the nine meiotic cases, a minimum of 56% (5/9) and a maximum of 100% (9/9) could have arisen from maternal meiosis I errors, 0-44% from paternal meiosis I errors, and 0-22% from maternal or paternal meiosis II errors. Table VI shows that in large, informative, Down Syndrome parental origin studies, 68% of errors are from maternal

TABLE XIV

SUMMARY OF RESULTS OF ORIGIN STUDIES
AUTOSOMAL TRISOMY MOSAICS

Reference	Chromosome	Origin				<u>Mitotic or Meiotic II</u>	
		<u>Meiotic</u>		Unknown			
		Mat I	Mat or Pat I		Mat	Pat	
Hassold, 1982	#4			1			
Hassold, 1982	#16	2					
Sanchez, et al. (1982)	#9		1				
Niikawa, 1977	#22	1					
Harris, et al. (1982)	#21					1	
This Study	#21	2	1	1	2	—	
Subtotal		5	2	2	2	1	
TOTAL:			9 MEIOTIC		3 MITOTIC OR MEIOTIC II		

meiosis I, 13% from paternal meiosis I, and 19% are from meiosis II. These ranges of types of errors are consistent with what would be expected if there were loss of a #21 chromosome from a random group of trisomy 21 cases. The 25% (3/12) of the cases which are the result of either meiosis II or mitotic errors are also consistent with the expected contribution of meiosis II errors if all the mosaics had been the result of meiotic errors. Therefore these results give no evidence for the assumption that trisomy 21 mosaics come from a mitotic error in a normal zygote. Many more cases need to be studied to get a large enough sample size to see if there are more than the expected number of meiosis II or mitotic errors. A difference between the observed and expected numbers of these cases would give an estimate of the frequency of mitotic errors among mosaics. However, as shown by Tables V and VI, the amount of contribution of meiosis II errors has varied among different laboratories. This makes a precise analysis very difficult.

Two of the seven definitely mosaic trisomy 21 cases which were a part of this study were atypical Down mosaics. Case 7 had a typical trisomic cell line, but all her cells which had only two #21 chromosomes had only one X chromosome so that her karyotype was

45,X/47,XX,+21. She was originally described by Cohen and Davidson in 1972. They suggested that the most likely explanation for her unusual double aneuploidy was that she arose as a 47,XX,+21 zygote with later simultaneous loss of an X and a #21, probably in a lymphocytic precursor cell. This hypothesis could have been confirmed if she had had three different 21s in her trisomic cell line. She may have been among the 19% of Downs which result from meiosis II errors, or her two very similar 21s could have been inherited independently from her parents. Unfortunately, no conclusions can be drawn based on her quinacrine heteromorphisms.

Case 4 had no phenotypic signs of Down Syndrome when he was born. His chromosomes (which revealed an extra #21 in some cells) were studied only as an aid in diagnosing his leukemia. Down Syndrome patients generally are about twenty times more likely to have leukemia than normal members of the population. Kardos, et al. (1983) found that 2.2% (17/756) of children with leukemia in Hungary had Down Syndrome. All were over one year of age. Fifteen had acute lymphoblastic leukemia and two had acute myeloblastic leukemia. Ten died within two months of diagnosis and initiation of treatment, and all had died by five years. In contrast to this low remission rate and poor survival is a benign

leukemoid reaction with spontaneous resolution which is sometimes seen in newborn Downs and can be difficult initially to distinguish from acute myeloblastic leukemia. Four cases of transient leukemoid reactions have been reported recently in mosaic Down patients. Brodeur, et al. (1980) reported the case of a phenotypically normal newborn boy who had 100% trisomy 21 in 50 blood cells examined at three and eight days and 22 bone marrow cells examined at 22 days. Fibroblasts at eight days had only 4% trisomic cells. At three months, only 24% of 75 blood cells were trisomic while no trisomy was found in 34 bone marrow cells. Brodeur's patient was reported by Weinberg, et al. (1983) to have had normal development with no signs of Down Syndrome at nine months of age. Weinberg, et al. described another phenotypically normal mosaic trisomy 21 neonate. At twelve days, she had 10% trisomic lymphocytes (50 cells). The trisomic cell line persisted until 31 months of age when 3% (3/100) cells were trisomic. At one year, no trisomic cells were found in 13 bone marrow cells or 85 skin fibroblasts.

An additional two cases were described briefly by Seibel, et al. (1983). One patient had a persistent trisomy 21 clone at age five years, while the other had no trisomic cells by the age of six months.

Of the three meiosis II or mitotic cases in Table XIV, only one (this study, Case 4) has typical Down Syndrome while the case in Harris, et al. (1982) was the phenotypically normal mother of three Down children and Case 5 (this study) had neonatal leukemia but no dysmorphic signs of Down Syndrome. Assuming that the mosaic abortuses studied by Hassold (1982) and Niikawa (1977) would have been grossly abnormal and including Sanchez, et al. (1982)'s trisomy 9 mosaic and the five typical Down Syndrome cases of this study, only one of ten cases (10%) is possibly the result of a mitotic error and it could easily be the result of a meiosis II error.

The results in the families with more than one trisomy 21 child were, however, not what would be expected if the children were the result of chance, independent occurrence of Down Syndrome. Two of the four children were the result of apparent maternal meiosis II errors. This compares with approximately 20% meiosis II errors in non-familial Downs. While the 50% meiosis II error rate in these two families could be ascribed to the small sample size, it is more significant when added to the two sibs in Magenis and Chamberlin (1981) (Cases 27 and 32). Both daughters in that family appeared to be the result of maternal

meiosis II errors. From Table VI it can be seen that usually only 14% of trisomy 21 is the result of such errors. For two such events to occur twice would be expected in only 1.8% of all families with two Down children.

A possible explanation for this high rate of "maternal meiosis II" errors is that the mothers are themselves mosaics of the type AB/ABB. They, like the case of Harris, et al. (1982), could have a trisomic cell line with two copies of one of the chromosomes of the normal cell line. Because the women are phenotypically normal it is plausible that they began life as normal zygotes who later had a mitotic misdivision which led to a trisomic cell line in their germ cells and, possibly, in other tissues. They would then have some trisomic "ABB" oocytes. After secondary non-disjunction, two-thirds of the abnormal eggs would be "AB" and one-third would be "BB". The one-third who had two copies of a single #21 would later be interpreted to be the result of a maternal meiosis II error. The chance of two sibs of a mosaic mother having the "BB" pattern would be 11% (1/9), considerably greater than the 1.8% probability if the sibs were chance events.

In conclusion, it appears to be likely that most

phenotypically affected Down Syndrome mosaics may come from mitotic loss of a #21 chromosome from an originally trisomic zygote. Many more cases will have to be examined to calculate a contribution by mitotic errors of normal zygotes. Phenotypically normal mosaics, such as the parents of more than one trisomy 21 child, may, however, usually result from mitotic gain of a #21 in a cell line of a normal embryo.

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ADDENDUM

Niikawa and Kajii published the results of origin studies of five cases of mosaic Down Syndrome in the January, 1984, American Journal of Human Genetics. Three subjects who had three different #21 chromosomes in their trisomic cell line, started as trisomic zygotes. Two other subjects (including one added in proof) could have resulted from either a diploid or a trisomic zygote.

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