Am J Hum Genet 34:125-133, 1982

Parental Trisomy 21 Mosaicism

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SUMMARY

A family with three children with trisomy 21 in which the mother is a phenotypically normal, trisomy 21/normal mosaic was studied. Chromosome 21 fluorescent heteromorphisms were used to document that two of the three number 21's in two of the Down syndrome offspring were of maternal origin. Five cytogenetic surveys in which both parents of a child with trisomy 21 were studied have been reviewed. From these data, it is estimated that 3% of couples producing a child with trisomy 21 can be explained by parental mosaicism. From 17 informative sibships, with one parent mosaic, the segregation ratio was estimated to be 0.43 ± 0.11.

INTRODUCTION

The observation of three siblings with Down syndrome due to trisomy 21 prompted a study of both parents. The mother was shown to be mosaic, with approximately 10% trisomy 21 cells. For counseling purposes, we wished to consider the contribution of the problem of mosaicism to the recurrence risk for trisomy 21. It has been suggested on the basis of dermatoglyphics that 10% of parents of children with Down syndrome are mosaic [1]. Data were compiled from the literature for a crude estimate of prevalence of parental mosaicism in families of children

Received February 14, 1981; revised April 13, 1981.

This research was supported in part by grants from the Carrie J. Loose Trust, the Victor Speas Trust, and funds from the Children's Mercy Hospital; and by grant HD07997 from the National Institute of Child Health and Human Development and by Mental Retardation Training Grant MCT-000920-11-1 from the Maternal and Child Health Service, National Institutes of Health.

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with trisomy 21. Family studies were tabulated for segregation analysis of trisomy 21/disomy 21 with one parent mosaic.

MATERIALS AND METHODS

Chromosomes were prepared for study from peripheral blood cultures of seven members of 3 generations of this family using standard techniques. In addition, the proband (individual III-4) (fig. 1) and both her parents were studied in skin fibroblast cultures from punch biopsies from the forearm cultured in basal medium (Eagle's) supplemented with 10% fetal calf serum and examined at the sixth passage. The chromosomes were examined in a variety of ways: Giemsa staining, trypsin G-banding [2], and quinacrine mustard staining [3]. The fluorescent chromosomes 21 from each person were printed at six to eight different exposure times to maximize detection of centromeric, short arm, and satellite variations [4].

Family reports from the literature were tabulated for segregation analysis by the Weinberg sib method [5]. Survey data from parents of single children with Down syndrome whose mothers were under 30 years of age were also compiled.

RESULTS

The origin of the extra chromosome 21 in two of the affected offspring can be clearly traced to the mosaic mother (fig. 1). The first child with Down syndrome

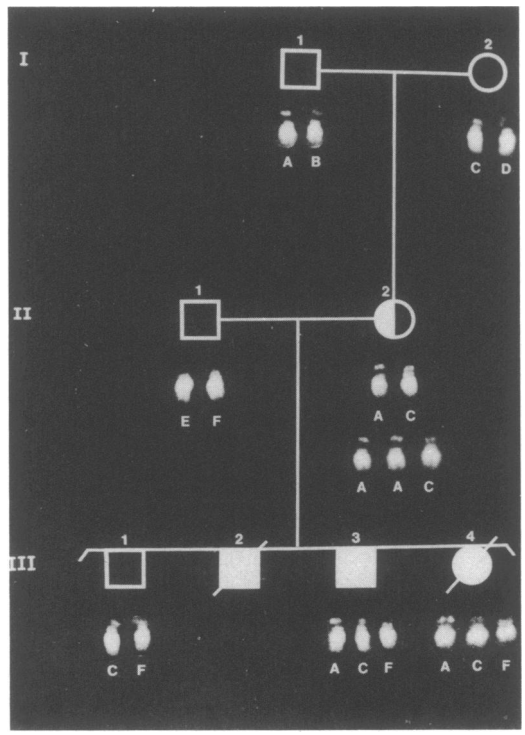

FIG. 1.—Inheritance of chromosome 21 in this family. Representative chromosomes 21 from the grandparents are labeled *ABCD*; the father, *EF*. Each chromosome is morphologically distinct.

was studied in 1972; unfortunately, the preparations were unsatisfactory for quinacrine fluorescence. In the two offspring suitable for study, each received two different number 21's from the mosaic mother and one number 21 from the father. The mother's trisomic cell line accounts for approximately 10% (six of 62 cells) of leukocytes. The mosaicism was confirmed in skin fibroblasts from the mother (four of 77 cells). In the trisomic cell population, there were two identical chromosomes 21 that were of grandpaternal origin; the third chromosome 21 was of grandmaternal origin. The father's leukocytes and skin fibroblasts were normal. Having counted 20 leukocyte metaphases, we can exclude 14% mosaicism or greater in the father with .95 confidence [6]. The proband demonstrated a heritable fragile site at band 16q22 in 15% (six of 40 cells) of lymphocyte cells, while the mother had the same fragile site in 20% (10 of 50 cells) of lymphocytes [7]. The proband also had "satellites" on the short arms of one of her number 17 chromosomes, as did her father [8].

Twenty-six families in which one parent was a normal/trisomy 21 mosaic have been described (table 1). In six families, there was paternal mosaicism; in 20 families, the mosaicism was maternal. In these 26 families, the mean age of the father at the birth of the first affected child was 25.2 years, while for the mothers, it was 26.7. The mean maternal age does not differ significantly from 25.8 years, which is the mean of all females giving birth in the United States ($t = 0.709$, $P = .24$) (K. Wright, U.S. Census Bureau, personal communication, 1976). On the other hand, the average age of the grandmothers (mothers of mosaic offspring) was 30.1 years, which is significantly different ($t = 2.66, P = .004$). There is also a difference between the mean ages of the fathers and grandfathers, ($t = 2.77$, $P < .01$) in which the mean age of the fathers is 25.2, and the grandfathers, 33.5 years.

In the material collected, there were only 17 sibships with informative data for segregation analysis. Because of the size of the sample, a relatively rapid, but statistically efficient Weinberg sib method was chosen. The probability that an affected individual is a proband π was estimated to be .8. The segregation ratio $\hat{p}$ was estimated at .43 $\pm$ .11. This cannot be distinguished from either .25 or .5 (see APPENDIX).

Table 2 shows the results of surveys of the parents of a single child with Down syndrome. Out of 221 couples, seven individuals (1.6%) were shown to be mosaic in either leukocytes, fibroblasts, or both cell types. There were six mosaic parents on whom analysis was performed on both lymphocytes and fibroblasts in which the number of each kind of cell counted as well as the percent mosaicism given. The proportion of mosaic cells in the two types of preparations was the same when compared by a Fisher exact test.

DISCUSSION

Understanding the mechanism that gives rise to more than one child affected with Down syndrome in a family (a multiplex sibship) is necessary for estimation of the recurrence risk in that family. Investigators in human cytogenetics have considered the following: (1) parental translocation, (2) parental mosaicism, (3) a

TABLE 1

FAMILY INFORMATION ON TRISOMY 21 MOSAICISM

MOSAIC PARENT	AGE AT BIRTH OF AFFECTED CHILDREN	TRISOMIC CELLS: LYMPHOCYTES No. counted	LYMPHOCYTES % trisomic	SKIN FIBROBLASTS No. counted	SKIN FIBROBLASTS % trisomic	OFFSPRING: Probands	OFFSPRING: Down syndrome	OFFSPRING: Normal	GRANDMATERNAL AGE AT BIRTH OF MOSAIC PARENT	GRANDPATERNAL AGE AT BIRTH OF MOSAIC PARENT	REFERENCE
Mother	22, 24	166	5	...	...	1	1	0	39	45	[9]
Mother	28	80	14	...	...	1	0	0	40	42	[10]
	...	36	11	...	...	...	...	...	...	...	
Mother	25	16	25	54	20.4	1	0	0	27	32	[11]
Father	21, 25	20	0	93	7.6	1	1	1	35	35	[12]
	...	...	...	50	4*	...	...	...	...	...	...
Father	21	50	6	...	...	1	0	0	20	30	...
Father	30	43	4.7	50	4	1	0	0	19	22	...
Mother	39	45	22	...	...	2	0	4	37	...	[13]
Mother	33	39	10	...	...	1	0	6	...	...	[14]
Father	23	60	6.7	...	...	1	...	...	31	36	[15]
Mother	20, 29	30	0	100	13	1	1	0	22	28	[16]
	...	100	3	...	...	...	...	...	...	...	...
Mother	22, 24, 24	208	0	688	0.87	2	1	0	29	...	[17]
Mother	24, 27, 29	200	6	...	...	3	0	0	26	33	[18]
Father	30	100	6	...	...	1	0	1	32	33	[19]
Mother	24, 26	...	2.6	...	...	...	...	...	44	...	[20]
Mother	27	...	25.5	...	...	...	...	...	27	...	...
Father	26	...	6.7	...	...	...	...	...	31	...	...
Mother	...	...	2	...	...	...	...	...	...	...	...
Mother	27	...	21.5	...	...	...	...	...	30	...	...
Mother	32	...	4.6	...	...	...	...	...	...	...	...
Mother	35	...	22.5	...	28.6	...	...	...	37	...	...
Mother	32, 34	...	2.2	...	...	...	...	...	18	...	...
Mother	25, 29	...	7.8	...	...	...	...	...	19	...	...
Mother	19	...	27	8	75	2	1	0	...	...	[21]†
Mother	26	...	10	...	...	1	0	1	...	...	[22]
Mother	32	155	10	...	...	1	...	1	34	...	[23]
Mother	17	105	16	50	18	1	0	0	39	...	[24]
Mother	25, 28, 29	62	10	77	5.2	1	2	1	26	33	
Total						23	7	15			

* Testicular tissue.

† Original report indicates two affected siblings; Weinstein and Warkany [22] in a personal communication from Smith indicate that there are now three affected sibs.

TABLE 2

RESULTS OF CYTOGENETIC SURVEYS ON PARENTS OF TRISOMY 21'S

No. couples screened	No. individuals with mosaicism	No. lympho-cytes counted	% mosaicism	No. fibro-blasts counted	% mosaicism	Reference
21	2	43	4.7%	?	0?	[25]*
		100	2%	?	0?	...
57	0	?	...	?	...	[26]*
70	4	20	0%	93	7.6%	[12] and Hsu†
		50	6%	...	...	...
		43	4.7%	50	8%	...
		...	...	...	...	...
73	1	30	0%	...	...	[16] and Mikkelsen‡
		100	3%	100	13%	...
Total 221	7 (3.2%)					

* No information supplied concerning the no. metaphase spreads routinely counted.

† Source: L. Y. E. Hsu, personal communication, 1977. Hsu routinely counted between 20 and 50 metaphase spreads per parent. (This would exclude between 14% and 6% mosaicisms or greater with .95 confidence in the parents who did not exhibit mosaicism.)

‡ Source: M. Mikkelsen, personal communication, 1976. Mikkelsen routinely counted 50 metaphase spreads per parent. (This would exclude 6% mosaicism or greater with .95 confidence in the parents who did not exhibit mosaicism.)

structural rearrangement or heteromorphism of the chromosomes distorting the meiotic process in such a way that nondisjunction is more likely to occur, and (4) a Mendelian gene producing a greater risk of nondisjunction. Translocations and other major chromosomal rearrangements should be detectable by the techniques most cytogenetic laboratories now use. A major gene producing a greater risk of nondisjunction should be detectable from a study of pedigrees and populations. If this gene were inherited as an autosomal dominant, then there should be families with multiplex sibships in several generations. If it were recessive, one would expect multiplex families to be more prevalent in a population with a high inbreeding coefficient. An increased incidence of Down syndrome has been reported in a highly consanguineous population [27]. We conclude that in the absence of a translocation, parental mosaicism is the most likely explanation.

Parental mosaicism, to be of reproductive significance, would have to involve germinal cells. A parent could be mosaic in either the germinal line or in both germinal and somatic cell lines. The detection of somatic mosaicism would depend upon the proportion of mosaic cells, the tissues studied, and the number of cells counted. From the data we have analyzed, fibroblast studies do not seem to have an advantage for detecting mosaicism over peripheral leukocytes. When the proportion of mosaic cells is low, the distribution of cells with 47 chromosomes in a sample of any given size should follow a Poisson distribution. There would then be a number of samples of a fixed size showing no abnormal cells. The previous reports of better success from fibroblasts probably represents the effect of counting an additional sample.

The mechanism of production of mosaicism is not known. However, the mean grandmaternal age at the birth of the mosaic parent of 30.1 years and the mean

grandpaternal age of 32.6 years suggest a possible age-dependent factor in the genesis of mosaics. This led Richards [20] to suggest that the mosaic parent may originate from a nondisjunctional error in parental gametogenesis, resulting in a trisomy 21 zygote; the trisomic zygote would then lose the extra chromosome from one cell line in an early mitotic division. This hypothesis requires two nondisjunctional errors: the first in parental gametogenesis and the second in embryonic mitosis. This would result in a trisomic cell line in which either the three chromosomes 21 are different (first meiotic error) or two are identical (second meiotic error). A less complex explanation would require the mosaic to originate from a diploid zygote undergoing a mitotic nondisjunctional error to produce the trisomic cell line; two of the chromosomes 21 would always be identical. Our data is more consistent with the latter theory; since the percentage of trisomic cells is low, a second meiotic error in the former theory cannot be ruled out.

Attempts have been made to estimate the prevalence of parental mosaicism as a cause of Down syndrome. Study of dermatoglyphic patterns initiated by Penrose led to the estimate that 10% of mothers and 1% of fathers were mosaic [28]. After studying dermal patterns of parents of individuals with Down syndrome and controls, Priest et al. suggested that 19% of trisomy 21 Down syndrome infants could be due to parental mosaicism (11% of mothers and 8% of fathers) [1]. In the surveys we have reviewed, seven mosaics among 442 parents screened, (1.6%) or 3.2% of couples, is a much lower estimate than that from either of the dermatoglyphic studies. No studies using both techniques have been reported, but we would expect that the lower estimate would be confirmed. Perhaps there is a relationship between the liability for nondisjunction and the dermatoglyphic microsymptoms. The prevalence of chromosome 21 mosaicism in a random normal population is not known, as most of the newborn surveys do not count a large enough cell sample. In 2,404 amniocenteses for advanced maternal age, there was one mosaic, but usually only 15 cells were counted [29].

Recurrence risks depend upon the extent of gonadal mosaicism. There may be germinal without somatic mosaicism, which could account for the multiplex sibships observed by Frohlich et al. [30] and Dhadial and Pfeiffer [31]. Hartl's models are probably appropriate for counseling in this situation. These include a synchronous, symmetric, dichotomous model with a constant rate of mutation that is similar to the models used in bacterial populations, as well as germinal cell models with and without gametic accumulation. Probabilities for a number of sibship configurations and cell divisions within the gonad are given [32]. This risk approaches a maximum of .5 as the number of affected increases but also depends upon the number of normal offspring. In the mouse with gonadal mosaicism at the albino locus, the mutant proportion ranged from 11.7% to 91.2%, but was, on the average, close to 50% [33]. The results of our segregation analysis are very similar ($\hat{p} = 0.43 \pm 0.11$) and the mean close to .5, but the variability is large. It is clear that a proportion of the gonads of the parents may have a much higher proportion of germinal cells with 47 chromosomes. The data do not permit precision in the estimate of the recurrence risk for parents with somatic as well as gonadal mosaicism. Qualitatively, if we use the convention that a risk over 10% is a high risk, then at least that much information may be conscientiously given.

Counseling for families with Down syndrome offspring depends upon an adequate theoretical basis as well as empirical data. Traditionally, after the birth of the first affected child, a "doubling or trebling" of the risk for a mother of the same age has been given. This risk estimate was quoted by Penrose in 1956 [34] based on Oster's monograph that appeared in 1953 [35]. Both of these studies obviously appeared prior to the availability of human cytogenetic studies and include both translocation and standard trisomies in the recurrence risk estimates. Stene [36], using cytogenetically confirmed cases of trisomy 21 and excluding known parental mosaic cases, estimated the recurrence risk for women who had their first affected child before age 30 to be between 1% and 2%. The recurrence risk was not increased over the general population if the first affected child was born when the mother was over age 30. Uchida [37], in extensive family studies, observed a risk of recurrence for trisomy 21 of 1:57. Milunsky [38] discussed national data culled from 1,663 amniocenteses and reports a 1:97 risk of recurrence. Golbus et al. [29] found the risk 1:67 for mothers over age 35 (2,404 amniocenteses) and 1:240 for younger mothers with previous trisomy 21. At the present time, we would estimate the recurrence risk to be between 0.3% and 4.6% from the segregation analysis and the survey. There are two areas that may provide more precise risk estimates: (1) the risk of producing a child with Down syndrome appears to be higher than previously believed since a higher frequency of affected has been found through the use of prenatal diagnosis in second trimester studies [39]; and (2) through the use of chromosome 21 fluorescent heteromorphisms. It is often possible to identify both the parent in whom the meiotic error occurred and the stage of meiosis [40]. This information will eventually provide recurrence risk data for trisomy 21 based on parental origin of the extra chromosome as well as stage of meiosis in which the nondisjunctional event occurred. If a consistent pattern is found, hypotheses for the production of nondisjunction might be testable and the likely origin of mosaicism established.

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APPENDIX

SEGREGATION ANALYSIS USING THE WEINBERG PROBAND METHOD

SUMMARY TABLE BY FAMILY SIZE OF SIBSHIPS WITH DOWN SYNDROME SEGREGATING

s	$S = \Sigma a(s-1)$	$R = \Sigma a(r-1)$	$A = \Sigma a(a-1)$	$1/C$	CS	CR
7	6	0	0	3.03	1.98	0
6	10	2	2	2.72	3.68	0.74
3	18	11	12	1.80	10.00	6.11
2	5	2	0	1.49	3.36	1.34
Total	39	15	12	...	19.02	8.19

NOTE: For each family—a = no. probands, r = no. affected, s = family size. The initial estimate of the segregation ratio is:

$$p_0 = \frac{\Sigma R}{\Sigma S} = \frac{15}{39} = 0.3846 \ .$$

The probability that an affected individual is a proband is:

$$\pi = \frac{\Sigma A}{\Sigma R} = \frac{12}{15} = 0.8 \ ,$$

$$1/C = 1 + \pi + \pi p \, (s - 3) \ .$$

The weighted estimate of p is:

$$\hat{p} = \frac{\Sigma CR}{\Sigma CS} = \frac{8.19}{19.02} = 0.4306 \ ,$$

$$v(\hat{p}) = \frac{\hat{p}(1 - \hat{p})}{\Sigma CS} = 0.01289 \ .$$