Maternal age and origin of non-disjunction in trisomy 21*

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SUMMARY The role of maternal age in chromosomal non-disjunction was investigated by studying 51 families in whom the origin of the meiotic anomaly had been identified. Results of this study were compared with previously published data. This comparison did not show any difference in mean maternal age, nor in distribution of maternal ages when the origin of non-disjunction was maternal, or paternal, or occurred at the first or second meiotic division. These results make questionable the role of maternal age in the birth of Down's syndrome children.

The increased risk of older women giving birth to a child with trisomy 21 has been known for many years.¹ Penrose and Smith² in their analysis of published reports concluded that there were two maternal age distributions: "one in which the maternal age is not a significant factor, and the other in which the maternal age is a fundamental influence". These comments were based on the observed livebirth incidence of trisomy 21. Until recently the majority of studies in this field have been based on similar birth data.³

With the advent of quinacrine fluorescence techniques, many chromosome variants have been revealed, allowing workers to evaluate parental and meiotic origin of individual chromosomes.⁴⁻¹²

The aim of this report was to study the origin of non-disjunction as a function of parental age using data from previous publications and our own results.

Material and methods

Seventy consecutive cases of trisomy 21 and their parents, referred to us from August 1977 to December 1978, were examined cytogenetically. These cases came from a large region, but mainly (52/70) from the department of Bouches-du-Rhône. During the same period, 28 255 liveborn children were reported by the Regional Public Health Service for the same department. The incidence of trisomy 21

can be estimated at 0.184% and it was thought that these cases represent all cases of trisomy 21 born during this period. Thus, in these cases (74.28% of the results presented are not based on biased ascertainment. However, it cannot be confirmed that the other cases outside this department (25.72%) represent all the cases in the region.

Slides were stained with quinacrine mustard and the structural variants were scored as described previously.¹² Fifty mitoses were analysed in all parents; the karyotypes were consistently normal and in particular no parental mosaics were observed.

In 51 of the families two independent observers agreed as to the origin of the supernumerary chromosome 21. In the other 19 families the two observers disagreed in six cases, and in 13 cases they agreed that assignment of chromosomal origin was impossible.

Four groups were identified according to the parental (maternal or paternal) and meiotic (first or second division) origin of the non-disjunction. Since age distributions did not follow a normal curve, statistical comparison of the different groups was made using Wilcoxon's test and the χ^2 test. For the latter test we compared maternal ages by scoring ages as follows: <30 years and ≥ 30 years; <35 and ≥ 35 ; <38 and ≥ 38 ; <40 and ≥ 40 . This is in agreement with the hypothesis that two maternal age distributions exist.² Fathers were divided into two groups according to age, <40 years and ≥ 40 years old, as proposed by Erickson.¹³

Maternal age distribution in the general population was based on census data on maternal age at time of birth from August 1977 to December 1978.

*Supported by grants from INSERM (ATP No 37.70.69)

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Received for publication 19 November 1979

in the Bouches-du-Rhône department (28 255 live births). In this population the mean maternal age was 26.3 years.

Results

Informative results were obtained in 51 families and are shown in table 1a and b according to paternal and maternal age and as a function of the parental and meiotic origin of non-disjunction. The parental ages of the 19 other families are shown in table 2.

The mean ages of the mothers and fathers of the informative and non-informative families do not differ significantly, neither do the age distributions. Thus the 51 families studied are representative of the 70 original families in terms of parental age.

Although to our knowledge the present study is the largest published one of its kind, the number of

TABLE 1b Cases of paternal non-disjunction: mean age of parents

Cases of paternal non-disjunction	Father	Mother	Case No
1st meiotic division failure			
Father : n = 7	21	28	42
$\bar{x}$ = 29.14	23	26	43
SEM = 2.96	26	25	44
	29	25	45
Mother : n = 7	29	26	46
$\bar{x}$ = 28.71	31	30	47
SEM = 2.15	45	41	48
2nd meiotic division failure			
Father : n = 3			
$\bar{x}$ = 34.00			
SEM = 7.09	25	28	49
	29	33	50
Mother : n = 3	48	40	51
$\bar{x}$ = 33.67			
SEM = 3.48			

TABLE 1a Cases of maternal non-disjunction: mean age of parents

Cases of maternal non-disjunction	Father	Mother	Case No
1st meiotic division failure	19	18	1
Father : n = 33	23	20	2
$\bar{x}$ = 32.54	23	20	3
SEM = 1.40	27	20	4
	28	22	5
Mother : n = 33	30	23	6
$\bar{x}$ = 29.78	26	24	7
SEM = 1.16	24	25	8
	26	25	9
	27	25	10
	28	26	11
	29	26	12
	38	26	13
	28	28	14
	36	28	15
	31	29	16
	34	29	17
	37	29	18
	29	32	19
	34	32	20
	26	33	21
	31	33	22
	35	33	23
	35	33	24
	43	33	25
	36	36	26
	35	37	27
	47	38	28
	35	39	29
	38	39	30
	60	40	31
	45	40	32
	31	42	33
2nd meiotic division failure	32	23	34
Father : n = 8	28	24	35
$\bar{x}$ = 30.62	34	26	36
SEM = 1.82	27	27	37
	31	29	38
Mother : n = 8	35	30	39
$\bar{x}$ = 28.75	37	34	40
SEM = 1.70	21	37	41

TABLE 2 Cases where the origin of the supernumerary chromosome is not known

	Age of father	Age of mother
	44	30
	32	37
Families where observers disagreed	36	31
n = 6	43	42
	28	21
	34	29
	34	37
	42	29
	28	27
	29	25
Inconclusive families	24	22
n = 13	36	24
	24	24
	51	37
	34	33
	31	31
	54	38
	19	17
	24	23
	$\bar{x}$ = 34.05	$\bar{x}$ = 29.32
	SEM = 2.15	SEM = 1.56

persons in certain groups is rather small. Accordingly, we combined our results with those reported by Magenis *et al*¹⁰ and Hansson and Mikkelsen¹¹ in order to yield more valid statistical analyses.

Regardless of whether non-disjunction is of paternal or maternal origin, there is a shift in the age distribution of mothers of trisomy 21 children with respect to the general population. There is an excess of older mothers at the birth of affected offspring (table 3; fig 1).

There was no significant difference between the maternal ages in groups where non-disjunction was of either paternal or maternal origin (table 4, 5).

TABLE 3 Distribution of maternal ages in data compiled from Magenis *et al.*¹⁰ Hansson and Mikkelsen,¹¹ and the present study

	Distribution of maternal age						
	≤ 19	20-24	25-29	30-34	35-39	40-44	> 45
Maternal origin of non-disjunction n = 80	2.5%	22.5%	30.00%	23.75%	10.00%	10.00%	1.25%
Paternal origin of non-disjunction n = 24	0	25.0%	37.5%	16.66%	8.33%	12.5%	0
General population n = 28 255 (mean maternal age 26.3 years)	5.7%	32.7%	37.9%	17.4%	4.9%	1.3%	0.1%

FIGURE Distribution of maternal age according to the origin of the non-disjunction.

TABLE 4 Expected and observed number of mothers according to age group and origin of non-disjunction

Mothers' age	Maternal non-disjunction		Paternal non-disjunction	
	Observed	Expected	Observed	Expected
< 30	44	45.38	15	13.62
≥ 30	36	34.62	9	10.38
< 35	63	63.07	19	18.93
≥ 35	17	16.93	5	5.07
< 38	67	66.15	19	19.85
≥ 38	13	13.85	5	4.15
< 40	71	70.76	21	21.24
≥ 40	9	9.24	3	2.76

TABLE 5 Statistical study with data compiled from Magenis *et al.*¹⁰ Hansson and Mikkelsen,¹¹ and the present study

Meiotic origin			
	1st division		2nd division
	Father	Mother	Father
Paternal origin	n = 15 $\bar{x}$ = 29.66 SEM = 1.85	n = 15 $\bar{x}$ = 28.93 SEM = 1.93	n = 9 $\bar{x}$ = 30.44 SEM = 2.32
Maternal origin	n = 65 $\bar{x}$ = 32.36 SEM = 1.10	n = 65 $\bar{x}$ = 30.00 SEM = 0.84	n = 15 $\bar{x}$ = 31.2 SEM = 1.42

When paternal age was used as a basis for comparison, we found no significant difference either (table 6).

Maternal age did not vary significantly when the stage of meiotic failure was examined, that is, there was no maternal age difference between first and second division meiotic failure (table 7).

Identical results (no significant difference) were obtained when maternal age ranks were compared as a function of the origin of non-disjunction using Wilcoxon's test, regardless of the arbitrarily chosen cut-off age.

In other words, although the distribution of ages of mothers at the birth of a child with trisomy 21 is

TABLE 6 Expected and observed number of fathers according to age group and origin of the non-disjunction

Fathers' age	Maternal non-disjunction		Paternal non-disjunction	
	Observed	Expected	Observed	Expected
< 40	69	68.4	20	20.6
≥ 40	11	11.6	4	3.4

TABLE 7 Expected and observed number of mothers according to age group and meiotic failure

Mothers' age	Origin of non-disjunction			
	Maternal		Paternal	
	1st division	2nd division	1st division	2nd division
< 38	53 (54.4)	14 (12.6)	11 (12.8)	8 (7.2)
≥ 38	12 (10.6)	1 (2.4)	4 (3.2)	1 (1.8)

different from the distribution of ages of mothers at the birth of normal children, this difference exists whether or not non-disjunction occurred in the mother, both in our data and in previously reported data.

Discussion

Our data are consistent with the null hypothesis that there is no difference in mean maternal age among the four groups of origin. It is possible that such a conclusion could be false because of a type 2 error. The reasons for such a situation are twofold. Firstly, there is an absence of trisomy 21 children born to older mothers in our data which masks the effect of maternal age. In our sample 21.56% of mothers were above 35 years of age, versus 13.63% in the data of Hansson and Mikkelsen,¹¹ and 25.8% in the data of Magenis *et al.*¹⁰ Our maternal age distribution does not differ from that of the data previously reported, and it is in agreement with published results showing that the maternal age of mothers of affected children is decreasing.¹⁴⁻¹⁹ Secondly, our sample is perhaps too small for the expected difference to be seen. If paternal origin of a supernumerary chromosome 21 is independent of maternal age, it would be expected that the distribution of maternal ages in this population would be the same as in the control population. Accordingly, the expected proportion of mothers above 35 years would be 6.3%. In the data compiled, when the origin of non-disjunction was maternal, the proportion of mothers above 35 years was 21.25%. In view of this discrepancy it can be estimated that our sample size would have to be twice as large in order to show a difference of this magnitude, choosing a low type 2 error.

Nevertheless, the fact that we did not observe any difference in the present sample merits a few comments. Concerning cytogenetic investigations, the following point should be made. The origin of non-disjunction is determined as a function of chromosomal morphology and possible chromatid exchanges involving the short arms are not taken into account. Although the latter event occurs infrequently,²⁰ its existence is a confirmed reality.²¹⁻²² Finally, Langenbeck *et al.*²³ and Jacobs and Morton²⁴ underlined the presence of bias when studying the transmission of chromosome markers. Although such bias exists regarding the meiotic timing of non-disjunction, it does not interfere when comparing the effects of maternal age in mothers who are and are not at the origin of the defect.

A significant correlation between ages of husbands and wives is well known and is verified in the present study. If paternal age were shown to have an effect

on the origin of non-disjunction, then it would be reasonable to expect that when non-disjunction is of paternal origin the mothers would be older than those in the general population simply because of the above correlation. However, this hypothetical situation assumes that the effect of paternal age is as intense as the effect of maternal age, but we know that this assumption is false. Even if paternal age is a factor involved,²⁵⁻²⁶ its effect is certainly a weak one. In our study only one father was older than 54 years and in this case non-disjunction was not of paternal origin.

When comparing distributions of maternal ages where non-disjunction occurred during the first meiotic division and those where non-disjunction arose during the second division, no significant differences were found.

Consequently, it should be concluded that either both first and second division non-disjunction are equally affected by maternal age, or that both events are unrelated to age, and that some other factor could be responsible for the higher maternal age observed at the birth of children with trisomy 21. These results lead to the necessity of questioning the role attributed to ageing of the ovum during first meiotic prophase in women. Indeed, since the incidence of offspring with trisomy 21 is higher in older women it is conceivable that maternal ageing could act, not during meiosis, but after fertilisation, by preferentially allowing development of trisomy 21 embryos. One point in favour of this hypothesis is that in our series the age of mothers with affected children was significantly higher than that of mothers in the general population, whether or not the non-disjunction was of maternal origin. The existence of such factors, as proposed by Erickson *et al.*,¹³ suggests that the relationship between maternal age and the incidence of trisomy 21 results from biased ascertainment which does not take into account spontaneous abortions of trisomy 21 embryos. In this respect it is important to note that the age of mothers of children with Klinefelter's syndrome is greater than the maternal age in the general population,²⁷ and paternal non-disjunction is involved in 39% of such cases.²⁸ In order to verify this suggestion, the age of women who have an early spontaneous abortion of a trisomy 21 fetus and the age of mothers who give birth to children with trisomy 21 could be compared. One would expect to find a significantly lower age in the spontaneous abortion group if the hypothesis were proved valid. Data published by Boue *et al.*²⁹ and Creasy *et al.*³⁰ do not verify this hypothesis. However, studies of this type involve women in whom pregnancy is detected and thus cannot take into account cases of very early abortion. Finally, comparison of populations

investigated at an interval of several years is difficult to perform because of the decrease in the mean maternal age and the presence of widespread contraception.

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