Origin of the additional chromosome in Down's syndrome: a study of 20 families

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SUMMARY In 20 families with a Down's syndrome child, heteromorphism of chromosome 21 was sought in the parents and proband. Information on the meiotic division and the parent of origin was given in 16 families, the majority of errors being maternal in origin, predominantly at meiosis I, though the paternal contribution is also appreciable. Probabilities of parental and meiotic division origin are calculated.

The first published studies tracing the origin of the extra chromosome 21 in Down's syndrome were those of de Grouchy¹ and Juberg and Jones,² who in two families where the rare 21p— Christchurch chromosome was present were able to show that non-disjunction had taken place at the second meiotic division in oogenesis. Subsequently, by quinacrine fluorescence techniques, the No 21 chromosomes were found to be polymorphic, showing inherited variants^{3,4} which had no abnormal effect on cell division. These techniques therefore allow the source of the extra chromosome in trisomic conditions to be traced in those matings where the chromosomes of the parents are sufficiently heterogeneous. With the refinement of technique by Overton *et al.*,⁵ in which the photographic exposure for each chromosome is standardised by the use of a fluorescent illuminator and automatic exposure control, and the entire chromosome is examined in sequential prints, more heteromorphism of slighter degree can be distinguished. This technique was applied in the present study.

Material and methods

Twenty families with a Down's syndrome child were examined and blood specimens were obtained from the 20 patients, their 20 mothers, and 20 fathers. These families were a random selection from those referred for cytogenetic diagnosis or counselling during the period of the study. Permission was obtained from the family doctor to visit the family and collect the specimens. From each subject 10 ml venous blood was taken into a heparinised bottle.

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As controls for the laboratory procedures, specimens were obtained from normal subjects with no family history of Down's syndrome, consisting of 21 members of staff from this Department and 39 other normal subjects.

The specimens were cultured by the procedure standard in this Department. Ten drops of whole blood were cultured in 5 ml media with filtered fetal calf serum. Phytohaemagglutinin was used as the mitogen. The cultures were incubated at 37°C for 70 hours and before fixation were treated with colcemid for 90 minutes, followed by 0.075 mol/l potassium chloride solution as hypotonic for 20 minutes. The cell suspension was fixed in 3:1 methanol:acetic acid mixture, with a minimum of four changes of fixative. Centrifugation was at 2000 rpm for five minutes. After overnight refrigeration, fresh fixative was again added and the cell suspension was dropped on to clean slides which had been kept in ethyl alcohol and wiped with a tissue before use. Slides were air or flame dried and labelled.

Slides were stained for a minimum of 15 minutes in 0.5% quinacrine dihydrochloride in methanol, washed, and mounted in distilled water. The cover slips were sealed to prevent evaporation. The slides were examined under an HBO 200 mercury vapour incident light source, FITC5 excitation filters, and a barrier filter OG515. Photography was by Kodak Tri-X Pan 35 mm film, with exposure times of 15 to 25 seconds. To maintain constant intensity of light, exposure time was increased as the lamp aged, and a new lamp was fitted every 180 hours. At least ten metaphase spreads were photographed per subject. Five to ten of the best quality negatives were printed, using sequential printing according to the method

of Overton *et al.*⁵ A piece of card with a frame cut in it was moved along a strip of photographic paper for serial exposures of each chromosome 21. The timing of exposures differed according to the intensity of the negative, but each chromosome was given a range of exposure from under- to over-exposed, usually at one second increments (fig 1). This enabled all the features and the banding patterns of each chromosome to be fully analysed.

Since the variant regions of the chromosomes cover a wide range of intensity and size and apparently vary continuously, five levels of intensity of fluorescence are recognised (Paris Conference, 1971). In the present study scoring was either positive (the brilliant and intense levels) or normal (the three lower intensity levels). The variants of the chromosomes 21 were scored in detail. The short arms, stalk, and satellites vary in size, and these were each scored on a four point scale (absent, small, medium, and large). The fluorescent intensity of the short arm and satellites were also recorded.

Using this procedure it is possible in the majority of cases to distinguish between the parental chromosomes. In a given mating the chromosomes are coded abcd if four can be distinguished and abcc or acbc if three (if two are morphologically identical), and from the code the origin of the additional chromosome in the trisomic child can be inferred. In the mating ab male by cd female, if the offspring has chromosomes abc, then non-disjunction must have occurred at the first meiotic division in the father.

If the offspring has chromosomes acc, then non-disjunction occurred at the second meiotic division in the mother. Trisomy arising from post-zygotic non-disjunction with subsequent loss of the monosomic cell line cannot be distinguished from that arising in the second meiotic division.

In practice, such an ideal mating in which four chromosomes can each be identified rarely occurs. However, a limited amount of information can be drawn from all matings. For example, in the mating ab male by cc female, a trisomy acc could be produced by non-disjunction at the first or second meiotic division in the mother, but not by non-disjunction at either division in the father. However, all unions contribute information on the probability of non-disjunction at a particular meiotic division in a given parent, and only in the case where all four parental chromosomes are identical are these probabilities equal. Hence the cases in a series can be combined, provided that they are not selected, to give estimates of the probability of non-disjunction occurring in each meiotic division in each parent. If p is the probability of paternal origin given non-disjunction, $1-p$ is the probability of maternal origin. If f is the probability of first division non-disjunction given maternal origin, $1-f$ is the probability of second division non-disjunction in the female. If $f+d$ is the probability of a first division error given paternal origin, then $1-f-d$ is the probability of second division non-disjunction in the male. From these the marginal probabilities of the four mutually

FIG 1 Seven different chromosome 21 (A to G) to illustrate the method.

exclusive types of non-disjunction can be calculated by straightforward maximum likelihood methods.

Results

The results of the investigation are set out in table 1. All families are given, whether interpretable simply or not, in order to avoid the bias that partial reporting of the data would introduce.

In nine cases the error could be definitely ascribed to one parent or another. Of these the mother was implicated in eight (for example, fig 2) and the

father in one (fig 3). The case where the male parent was definitely involved indicated error at the first meiotic division. Where the female was the source, in four the first meiotic division was indicated, in one the second division was indicated, and in three an error at either could have been responsible. In four cases all four parental chromosomes were morphologically identical. In the remainder, although the supernumerary chromosome might have been attributed to either parent, one or more of the four possible origins could be eliminated, and so some indication of origin was given. Error at the

TABLE 1 Observed mating types producing a Down's syndrome child, parental ages at time of birth, and conditional probabilities of sources of non-disjunction

Family No	Mating M × F		Affected child	Conditional probabilities				Paternal age	Maternal age
				M parent		F parent			
				Meiotic division 1	2	Meiotic division 1	2		
1	aa	ab	aabF	$\frac{1}{2}$	$\frac{1}{2}$	1	0	19	18
2	aa	ab	aabF	$\frac{1}{2}$	$\frac{1}{2}$	1	0	30	24
3	aa	bb	abbF	0	0	1	1	33	33
4	aa	bc	abcM	0	0	1	0	36	37
5	ab	aa	aabF	1	0	$\frac{1}{2}$	$\frac{1}{2}$	31	29
6	aa	aa	aaaF		Not informative			29	27
7	ab	ac	abcM	$\frac{1}{2}$	0	$\frac{1}{2}$	0	25	23
8	ab	aa	aabM	1	0	$\frac{1}{2}$	$\frac{1}{2}$	28	27
9	aa	bb	abbM	0	0	1	1	46	41
10	ab	cd	acdM	0	0	1	0	25	23
11	bc	aa	aabM	0	0	1	1	27	26
12	ab	cd	accM	0	0	0	1	28	26
13	aa	aa	aaaM		Not informative			46	42
14	aa	aa	aaaM		Not informative			41	40
15	aa	aa	aaaF		Not informative			27	24
16	ab	cd	acdM	0	0	1	0	28	35
17	ab	cd	acdM	0	0	1	0	22	22
18	ab	aa	aabF	1	0	$\frac{1}{2}$	$\frac{1}{2}$	31	29
19	ab	ac	abcF	$\frac{1}{2}$	0	$\frac{1}{2}$	0	33	28
20	ab	cd	abcM	1	0	0	0	24	32

FIG 2 A maternal first meiotic division is the source of the error.

FIG 3 Error at the paternal first meiotic division. The second maternal chromosome is excluded by its greater fluorescent intensity as well as its morphology.

first meiotic division in either parent was indicated in one case. In two cases the first meiotic division in the mother, or either in the father, was suggested. In three cases the derivation appeared to be from either the first meiotic division in the father or either division in the mother. Combined with the information on the definitive assignments, of the 16 unions each with four possible sources of error, there are 33 that can be definitely eliminated. Of these, 14 are the second paternal meiotic division, nine the second maternal, eight the first paternal, and two the first maternal meiotic division.

These results are summarised in table 1, together with the conditional probabilities for each case that the error in the child derived from non-disjunction in a given parent or division. From these the maximum likelihood estimates of the defined probabilities are calculated as: $p = 0.136 \pm 0.093$, $d = 0.119 \pm 0.072$, $f = 0.881 \pm 0.072$. From these the probability at each of the four sources is as follows.

Male first division	$p(f+d)$	0.136
Male second division	$p(1-f-d)$	0.0
Female first division	$f(1-p)$	0.761
Female second division	$(1-p)(1-f)$	0.103

It is interesting that d , the measure of sex variation in the probability of non-disjunction at a given division, is not significantly different from 0.

The parental ages are shown for the informative matings. Maternal errors definitely at first meiosis occurred at maternal ages of 22, 23, 35, and 37. The error at the maternal second meiosis occurred at a

maternal age of 26. The paternal age in the first meiosis error was 24. The definite maternal errors at either first or second meiosis occurred at maternal ages 33, 41, and 26. From the small number of specimens examined, little conclusive can be expected, but the results indicate that maternal meiosis errors occur at all ages and that there is no tendency for either late or early parental age to be associated with a specific error.

Discussion

Reports of similar studies summarised in table show that, as in the present series, the majority of errors are maternal in origin, predominantly at meiosis I. Studies which have given parental ages show that maternal meiosis I errors predominate at all ages.⁷ In the present series the ages of the parents responsible are distributed across the whole range from 18 to 41.

However, comparison of published series is not straightforward on account of the bias towards the detection of non-disjunction of events in the second meiotic division, as noted by Robinson.⁸ There are seven possible mating types:

$aa \times bb$
 $aa \times ab$
 $aa \times bc$
 $ab \times ac$
 $ab \times cd$
 $aa \times aa$
 $ab \times ab$

The information they give depends upon the karyotype of the offspring, and does not always allow definite assignment of the error to a given meiotic division or parent. Among studies up to 1976 which reported mating types, all of the second meiotic division errors were found from matings where only this event could be detected. Initially, because there were few recognisable variant types of chromosome 21, matings in which only second meiotic error could be detected were more frequent than those in which both first and second meiotic non-disjunction could be identified. However, the improved photographic technique of Overton *et al*⁵ has shown an increased degree of heteromorphism "which will markedly increase the success rate and permit valid unbiased conclusions".⁷ But unbiased conclusions will only come from the reporting and analysis of complete series as here. Hence the importance of using all available information from all available matings to calculate probabilities. There are relatively few published series where reporting is complete; hence comparison is only made with the series of 266 cases of trisomy 21 for which the

TABLE 2 Definite assignments of non-disjunctional events in standard trisomy 21

Reference*	1st meiotic division		2nd meiotic division		1st or 2nd meiotic division		No of informative cases
	Mat	Pat	Mat	Pat	Mat	Pat	
Bott <i>et al</i> (1975)			3	4			7
de Grouchy (1970)			1				1
Dutrillaux (1975)	1		1				2
Emberger and Taib (1975)			1				1
Giraud <i>et al</i> (1975)	1		1				2
Hansson and Mikkelsen (1978)	9	3	6	4	4		26
Hara and Sasaki (1975)	1		2	1	2		6
Juberg and Jones (1970)			1				1
Kajii and Niikawa (1973)	1						1
Kajii <i>et al</i> (1976)	3†						3
Licznarski and Lindsten (1972)	1						1
Magenis <i>et al</i> (1977)	23	5	1	2			31
Moore <i>et al</i> (1976)	1						1
Mutton (1973)			1				1
Niikawa <i>et al</i> (1977)†	3		1				4
Punnett <i>et al</i> (1973)	1			1			2
Robinson (1973)	4						4
Sasaki and Hava (1973)				1			1
Schmidt <i>et al</i> (1976)	22	1					23
Uchida (1973)				1			1
Wagenbichler <i>et al</i> (1976)	7	4	2	3	1	1	18
Present study	4	1	1		3		9
Total	82	14	22	17	10	1	146

* Full references available from the authors on request. † Spontaneous abortions.

matings are reconstructed by Hassold and Matsuyama.⁹ The present estimates of p, d, and f (0.136, 0.119, 0.881) are not significantly different from their respective estimates of 0.241, 0.003, 0.773. Comparison was by the log ratio test giving a value of $G = 2.94$, df 3, $p > 0.05$.

While caution is necessary in drawing together information on the origin of the third chromosome in Down's syndrome, the evidence appears overwhelming that, despite the bias towards detection of errors that occurred at meiosis II, most non-disjunctions occur at meiosis I, and with this the probability estimates agree. The finding that non-disjunction from the female parent does not occur at meiosis II with the same frequency as meiosis I is not surprising in view of the duration of the first meiotic stage in the female. First and second meiotic divisions may be influenced in quite different ways by quite different factors. The results so far also indicate that the male parent contributes the extra chromosome in an appreciable proportion of trisomy 21 cases, in the present study one out of 16, and in published material 29 out of 127, informative cases, though the precise probability remains to be established. Finally, it is not yet clear whether there is sex variation in the probability of non-disjunction at a given meiotic division, and this is a key question that remains to be answered for the information it will give on the mechanisms responsible.

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