A fluorescence polymorphism associated with Down's syndrome?

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SUMMARY Fluorescence polymorphism frequencies have been determined for a group of 85 Down's syndrome cases and 164 controls. For one class of polymorphism, that of positive satellites of chromosome 21, the frequency in the Down's cases was significantly higher than in the controls; the distribution of positive satellites in the mongols indicates that in the majority the extra chromosome arose by first meiotic non-disjunction. The possibility that positive satellites on chromosome 21 could be a causative factor in Down's syndrome is discussed, and the implications of this possibility on the assessment of the risk of producing a Down's child are examined.

Down's syndrome (mongolism) is the most common autosomal disorder in man; it affects around 1 in 600 newborns, causing a major social and economic problem. The elucidation of its aetiology, or of any predisposing factors is thus of considerable importance.

The chromosome involved in the trisomic state in mongolism, the number 21, has been found to be polymorphic for size and fluorescence intensity of the short-arm region, when stained with a quinacrine dye and viewed with blue light (Paris Conference, 1971). It was thought possible that one particular polymorphic type might be predisposed to meiotic non-disjunction, so that individuals with that type of polymorphic chromosome 21 would be at increased risk of having a Down's syndrome child. Ideally, we would have tested this hypothesis directly by examining the fluorescence polymorphisms of the parents of cases of Down's syndrome, their offspring, and control families. Access to sufficient affected families, however, proved difficult, so the problem was approached indirectly by analysing the fluorescence polymorphisms of mongols themselves together with those of matched controls. Certain of these matched pairs were also used in an investigation of satellite association, but the main results of that study will be published elsewhere. We report here on the frequencies of various types of fluorescence polymorphism of chromosome number 21 in Down's syndrome patients and controls, and present evidence for an increased occurrence of a particular kind of polymorphic 21 in affected individuals.

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Subjects and methods

Eighty-five individuals (50 male, 35 female) with trisomy 21 were used in the study; 79 were in institutions for the mentally subnormal and 6 (including four neonates) were in the general population. The age range was 0 to 59 years, with a mean of 37 years.

All institutionalized cases of Down's syndrome had a subnormal control from the same institution, matched for sex and age; the age difference between a Down's case and control was usually 5 years or less, never more than 10 years. The general population Down's cases had a similarly matched general population control. Lymphocyte cultures for fluorescence polymorphism analysis from these matched pairs were grown, processed, and examined together, to minimize scoring variation. All institutionalized Down's cases also had a general population control matched for age and sex, in case there were differences in polymorphism frequencies between the general population and institutionalized subnormals. These latter control cultures were not processed and examined at the same time as the Down's cultures.

All chromosome observations were made on lymphocytes from peripheral blood cultured by a modification of the whole blood micro method of Hungerford (1965). Slides for fluorescence analysis were stained for 8 minutes in a 0.5% solution of quinacrine dihydrochloride in deionized water, washed in running tap water for 3 minutes, rinsed briefly in, and then mounted in, deionized water.

Fig. 1 Chromosome 21, fluorescence intensity polymorphisms. (a) Normal short arms, positive satellites; (b) positive short arms, positive satellites; (c) normal short arms, normal satellites.

They were examined the same day, using blue light (BG12 exciting filter) and a fluorescence microscope fitted with a Ploem illuminator.

The fluorescence intensity polymorphisms vary over a wide range, apparently continuously. The report of the Paris Conference (1971) recognized five levels of fluorescence intensity, but deciding into which category the intensity of a polymorphism falls is not easy, especially at the lower intensity levels. There is also a certain amount of technical variation between slides which exacerbates the problem. For these reasons only two classes of fluorescence intensity were considered, positive (+) (the Brilliant and Intense levels, Paris Conference, 1971) and normal (–) (the three lower intensity levels) (Fig. 1).

The short-arm, gap, and satellites (Fig. 2) all vary in size and were scored on a four-point scale, absent (or apparently so), small, medium, and large. Less reliance, however, can be placed on the size data than on the intensity data, because the apparent size of a polymorphic region is not independent of its fluorescence intensity; while it is comparatively

Fig. 2 Chromosome 21, the three regions polymorphic for size.

easy to assess the size of a positive satellite, it is difficult to do so for a very pale one.

All scoring was done by one observer directly down the microscope.

Results

Tables 1, 2, and 3 show the results obtained. For many of the polymorphic categories the numbers are too small for adequate statistical analysis; an attempt has been made, using Fisher's exact test, but it is unlikely that any statistically significant differences in these categories between the mongols and controls have any biological significance in the aetiology of Down's syndrome.

In the case of those categories with adequate data, the two control populations were tested for homogeneity using a χ^2 contingency test, and where no difference was found the data were summed. In two

Table 1 Frequency of short arm polymorphisms of chromosome 21

	Small short arms		Medium short arms		Large short arms		Positive short arms	
	No.	Frequency	No.	Frequency	No.	Frequency	No.	Frequency
Subnormal controls	104	0.658	53	0.335	1	0.006	2	0.013
General population controls	122	0.718	47	0.276	1	0.006	2	0.012
Combined controls	226	0.689	100	0.305	2	0.006	4	0.012
Down's syndrome	188	0.737	65	0.255	2	0.008	2	0.008

Table 2 Frequency of gap polymorphisms of chromosome 21

	Absent gap		Small gap		Medium gap		Large gap	
	No.	Frequency	No.	Frequency	No.	Frequency	No.	Frequency
Subnormal controls	12	0.076	90	0.570	46	0.291	10	0.063
General population controls	14	0.082	96	0.565	55	0.323	5	0.029
Combined controls	26	0.080	186	0.567	101	0.307	15	0.046
Down's syndrome	15	0.059	165	0.647	68	0.267	7	0.027

Table 3 Frequency of satellite polymorphisms

	Absent sats.		Small sats.		Medium sats.		Large sats.		Positive sats.		Large positive sats.	
	No.	Frequency	No.	Frequency	No.	Frequency	No.	Frequency	No.	Frequency	No.	Frequency
Subnormal controls	1	0.006	75	0.475 ^a	76	0.481 ^b	6	0.038	16	0.101	4	0.025
General population controls			107	0.629 ^{a1}	56	0.329 ^{b1}	7	0.041	14	0.082	4	0.024
Combined controls							13	0.040	30	0.091 ^c	8	0.024
Down's syndrome			136	0.518	106	0.416	13	0.050	38	0.149 ^{c1}	9	0.035

The difference between a and a₁ is significant ($\chi^2=7.93$; $P\leq 0.01$).

The difference between b and b₁ is significant ($\chi^2=7.82$; $P\leq 0.01$).

The difference between c and c₁ is significant ($\chi^2=4.61$; $P\leq 0.05$).

classes only was there a difference between the control populations; the subnormal controls had a higher incidence of small satellites and a lower incidence of medium satellites than did the general population controls (Table 3). If the data for the two classes of polymorphism are summed, the evidence of 'small and medium' satellites in the two populations is virtually identical, and it may well be that the abnormal result is the result of scoring error. In any case, it is unlikely to be important in the causation of trisomy 21 as the mongol incidence for each class falls midway between those of the control populations.

In all other categories the control data appeared homogeneous and was, therefore, combined, the Down's data being tested against the results so obtained. A significant difference ($\chi^2=4.61$; $P\leq 0.05$) was found for only one class of polymorphism, that of positive satellites (Table 3). The frequency of such a polymorphism was 0.149 in the Down's cases and 0.091 in the controls. We have, in this laboratory, scored intensity variants of fluorescence polymorphisms for three other normal populations, in a total 742 individuals (Buckton *et al.*, 1976). The frequency of positive satellites on chromosome No. 21 found in that study was 0.096, not significantly different from the two control populations examined here. If all these control data are summed and tested against those from the Down's cases, the significance is now at the 1% level.

The frequencies of control genotypes for positive (+) and normal (−) satellites were tested against those expected from the Hardy-Weinberg Law (a binomial distribution). The data fit is extremely

good (Table 4) suggesting that the population is in equilibrium.

Discussion

Fluorescence polymorphisms of chromosome No. 21 have been scored on a sample of 85 Down's cases and 164 controls. Seventy-nine of these controls were subnormal individuals in an institution and 85 were from the general population. In only one category, that of positive satellites, did the frequency in the Down's cases (0.149) differ significantly from that of the controls (0.091). This control frequency is very close to that (0.096) of a larger population of normal individuals previously studied in this laboratory, suggesting not only a similarity in polymorphism frequency between these groups, but also a consistency in scoring, which is reassuring with so subjective a technique.

Mikelsaar *et al.* (1975) have also investigated the frequency of various polymorphisms in normal adults, Down's children, and subnormal children. No significant differences were observed for polymorphism of chromosome number 21, but the only category tested for satellites was 'marker brilliant' in which the satellites were as large as or larger than the short-arms. This seems to approximate to our 'large positive' class, in which no significant differences were found between the groups (Table 3).

The difference in frequency of positive satellites between mongols and controls in this study may well indicate an association between this polymorphic type and the tendency to non-disjunction. If the difference found is confirmed, then the implication is that positive satellites of the 21 are found in a higher frequency in parents of mongols than in the general population. Some evidence for this is to be found in an abstract of a recent communication by Bott *et al.* (1975), which reports a very much higher frequency of 'Q polymorphism' in Down's syndrome (33.3%) and their families as compared to the normal population (7%). Further evidence that this difference between Down's cases and the general population may have some biological significance comes from our

Table 4 Distribution of control genotypes, positive and normal satellites

		Observed	Expected
Controls			
n = 164	++	3	1.4
	+-	24	27
	--	137	135.6

+ = Positive.

− = Normal.

satellite association studies. While we find no overall difference between mongols and controls in the pattern of association of satellited chromosomes at metaphase, we do find that the 21's with positive satellites associate significantly less often than do those with normal satellites; this is in fact a general phenomenon for all acrocentrics, not just the 21's. The relation between satellite association and non-disjunction remains obscure but these results do indicate that the polymorphic character of a chromosome can affect its behaviour.

The greater frequency of positive satellites in the mongols could be accounted for under one of two hypotheses.

(1) The parent in whom the non-disjunction occurred could be a member of a subgroup with an inherent increased risk of producing mongol children, which is not a consequence of any particular polymorphic state, but which might be the result of the presence of gene(s) for non-disjunction, or a genetic predisposition for pregnancy at advanced maternal age. Individuals in such a subgroup would then have a higher frequency of positive satellites than the general population, but the tendency to non-disjunction would be the same for positive and normal chromosome No. 21. Non-disjunction could occur at either first or second meiotic division. One can calculate the polymorphism frequency in this subgroup from the frequencies in the Down's cases assuming that the normal risk parent does not belong to the subgroup. The distribution of the genotypes expected in the mongols under this hypothesis is shown in Table 5; it is obvious that the data fit the distribution expected on the basis of first meiotic non-disjunction extremely well.

(2) The other possibility is that the responsible parents have the same polymorphism frequency as

the general population, but that chromosomes with positive satellites have a higher chance of non-disjunction than those with normal satellites. This difference in probability would be about a factor of 2, as judged on the difference in frequency of positive satellites between Down's cases and controls. The calculation of expected genotypes is more complex than in the previous situation as there are unknown factors involved; the major problem is the absence of information on the relative tendencies of the two types of polymorphism and their combinations to undergo non-disjunction. One can say, however, that if second meiotic non-disjunction is the rule then one would expect more of the $+/+/-$ class in the mongols than the $+/-/-$ class, which we do not find in our data (Table 5). For first meiotic non-disjunction there are three unknowns; (a) the probability of a $+/+$ non-disjunction, (b) the probability of a $+/-$ non-disjunction, and (c) the probability of a $-/-$ non-disjunction. The differences between the probabilities a, b, and c, are almost certainly small and one can make various reasonable suggestions as to their possible values. If one substitutes the empirical relative values of $\times 4$, $\times 2$, and $\times 1$ to a, b, and c, respectively, then the expected distribution of genotypes fits that found very well (Table 5).

These possibilities are not mutually exclusive and it may well be that there are others that should also be considered. However, it appears from the distribution of genotypes of these mongols that many, if not most of them, were the results of first meiotic non-disjunction. We know from other studies (Bott *et al.*, 1975; Mutton, 1973; Sasaki and Hara, 1973; Uchida, 1973) that second meiotic non-disjunctions do occur, but their relative frequency compared with first meiotic non-disjunctions is yet to be ascertained.

Table 5 Distribution of Down's syndrome genotypes, positive and normal satellites of chromosome 21

Down's Syndrome	Hypothesis 1				Hypothesis 2					
	1st Meiotic non-disjunction		2nd meiotic non-disjunction		1st meiotic non-disjunction		2nd meiotic non-disjunction			
n = 85	Observed	Expected	Observed	Expected	Observed	Expected	Expected on assumed values of a, b, c*	Observed	Expected	Expected on assumed values of x, y†
+++	—	0.28	—	1.4	—	a	0.28	—	x	1.5
++-	5	4.8	5	13.8	5	9(a+2b)	3.85	5	10x	15
+--	28	27.5	28	6.4	28	81(2b+c)	28.8	28	10y	7.6
---	52	52.4	52	63.58	52	729(c)	52	52	100y	76

+ = positive
- = normal

a is probability of ++ non-disjunction
b is probability of +- non-disjunction
c is probability of -- non-disjunction
a > b > c

x is probability of ++ non-disjunction
y is probability of -- non-disjunction

*a = 4, b = 2m, c = 1 (suggested empirical values).
†x = 2, y = 1 (suggested empirical values).

Fig. 3 Distribution by maternal age, of births of Down's syndrome cases and general population controls.

Nor as yet do we know what proportion of Down's cases are due to paternal rather than maternal non-disjunctions. Originally, the existence of a pronounced maternal age effect in the incidence of Down's syndrome was taken to mean that most, if not all, causative non-disjunctions were maternal and the early fluorescence polymorphism evidence tended to confirm this (Licnerski and Lindsten, 1972; Robinson, 1973; Mutton, 1973). Recently, however, it has become apparent that paternal non-disjunction may be more important than previously thought. Bott *et al.* (1975) studied 59 Down's cases and their families; in 7 cases, the polymorphisms were informative and in 4 out of 7, the father had contributed the extra chromosome. This, however, may be an age-related phenomenon; while Bott *et al.* give no maternal ages for their informative cases, only 26 of their 59 Down's cases (44%) were born to mothers over 35 years. We have been able to obtain maternal ages for only 75 of our 85 Down's cases, but 51 (68%) were born to mothers of 35 and older. The maternal age distribution, compared with that of the general population controls is shown in Fig. 3. In view of the advanced maternal ages of our Down's cases we feel it is not unreasonable to assume that most of them have an extra maternal chromosome No. 21.

Assuming that 90% of these Down's cases have a maternal first meiotic non-disjunction as the origin of their extra chromosome No. 21, then on the first hypothesis the frequency of positive satellites on the 21's of their mothers is calculated to be 0.166 compared with the control frequency of 0.091. A woman with such a polymorphism would then have a risk of having a Down's child 1.99 times higher than a woman of the same age without this polymorphism. On the second hypothesis and with the suggested values of a, b, and c, women with both, or one, chromosome 21 with positive satellites would be,

respectively, about four times and twice as likely to have a Down's child as a woman of the same age without such a polymorphism.

The present data are obviously insufficient to choose between these two hypotheses. The satellite association results already mentioned do suggest, however, that the polymorphic character of a chromosome has some influence on its behaviour, and this might well include its tendency to undergo non-disjunction. Under either hypothesis, we would expect a higher than normal frequency of positive satellites on the chromosome 21 in the responsible parents of Down's children, but, if hypothesis 1 is correct, the genotypes in these parents should be distributed according to the Hardy-Weinberg Law, and dependent only on the polymorphism frequencies in the subgroup. With hypothesis 2, the distribution of genotypes depends not only on the frequencies of the polymorphisms, but on their relative probabilities to undergo non-disjunction.

Obviously more data need to be collected before we can be both certain of the causative association suggested here and define the additional risk that the possession of positive satellites may confer upon an individual, but we are hopeful that soon it will be possible to define a woman's risk of producing a Down's child with considerably more accuracy than from an estimate based on age alone.

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References

- Bott, C. E., Sekhon, G. S., and Lubs, H. A. (1975). Unexpected high frequency of paternal origin of trisomy 21. *American Journal of Human Genetics*, **24**, 20A.
- Buckton, K. E., O'Riordan, M. L., Jacobs, P. A., Robinson, J. A., Hill, R., and Evans, H. J. (1976). C and Q band polymorphisms in the chromosomes of three human populations. *Annals of Human Genetics*, **40**, 99-112.
- Hungerford, D. A. (1965). Leukocytes cultured from small innocula of whole blood and the preparation of metaphase chromosomes by treatment with hypotonic KCl. *Stain Technology*, **40**, 333-337.
- Licnerski, G., and Lindsten, J. (1972). Trisomy 21 in man due to maternal non-disjunction during the first meiotic division. *Hereditas*, **70**, 153-154.
- Mikelsaar, A. V. N., Kaosaar, M. E., Tuur, M. H., Viikmaa, M. H., Jalvik, T. A., and Laats, J. (1975). Human karyotype polymorphism. III. Routine and fluorescence micro-

- scopic investigation of chromosomes in normal adults and mentally retarded children. *Humangenetik*, **26**, 1-23.
- Mutton, D. E. (1973). Origin of the trisomic 21 chromosome. *Lancet*, **1**, 375.
- Paris Conference (1971). Standardisation in Human Cytogenetics. *Birth Defects: Original Article Series*, VIII: 7, 1972. The National Foundation, New York.
- Robinson, J. A. (1973). Origin of extra chromosome in trisomy 21. *Lancet*, **1**, 131-133.
- Sasaki, M., and Hara, Y. (1973). Paternal origin of extra chromosome in Down's syndrome. *Lancet*, **2**, 1257-1258.
- Uchida, I. (1973). Paternal origin of extra chromosome in Down's syndrome. *Lancet*, **2**, 1258.