Chromosome distribution studies in XXY karyotypes

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SUMMARY The method of 'generalised distances' was applied to characterise the relative position of the metaphase chromosomes in a population of XXY subjects (189 metaphases from 28 subjects).

The most striking observation was that the presence of an extra gonosome coincided with a disturbance of the normally stable centromere-centre distribution pattern. The distribution analysis gave no clear cut evidence for the induction of gonosomal trisomy XXY by chromosome association. No significant association was observed between X and X or X and Y but there was a smaller distance between X and Y in XXY karyotypes than in XY karyotypes.

As far as autosomes are concerned, the XXY karyotypes were characterised by a less central location of the acrocentrics without a clear decrease of association frequencies of these acrocentrics, and the C heterochromatin rich chromosomes were more often associated than in the XX and XY control populations.

These data do not support the idea that gonosomal trisomies result from chromosome associations, but favour the hypothesis that spindle degeneration as a result of intrafollicular ageing or C heterochromatin polymorphism may be responsible for non-disjunctions.

Different mechanisms have been considered to be responsible for non-disjunctions in trisomies. Non-disjunction may be induced by spindle degeneration as a result of intrafollicular ageing.¹ Close associations between chromosomes, on the other hand, were shown to induce trisomy 21, for example.² These associations are governed by the presence of rDNA connectives³ in combination with rRNA⁴ and proteins and by the existence of repetitive DNA families, for example in the C heterochromatin blocks of chromosomes 1, 9, 16, and Y.⁵

An analysis of the relative position of chromosomes in cells from trisomic patients and their parents would at least help to clarify the role of chromosome associations in the induction of trisomies. The determination of the centromere to centromere distances would show whether the chromosomes involved in the trisomy lie closer to each other or to some specific chromosome pair. The analysis of the centromere-centre distances would define whether the presence of an extra chromosome modifies the generally stable organisation of chromosomes as to the metaphase plate centre.⁵

We applied the objective method of 'generalised

distances'⁶⁻⁸ to characterise the relative position of the metaphase chromosomes in a population of XXY subjects (189 metaphases from 28 subjects).

Material and methods

Peripheral blood samples were taken from 28 XXY patients (mean age=24.2, range=0-61). The peripheral lymphocytes were cultured for 72 hours, fixed, and spread on slides.⁹ The metaphases were G banded and karyotyped (one subject, 12 metaphases; one, 11 metaphases; one, ten metaphases; one, nine metaphases; one, eight metaphases; eight, seven metaphases; eight, six metaphases; and seven, five metaphases).

The control groups consisted of two previously analysed populations^{5 8}: 100 metaphases from ten normal 20-year-old human males (ten metaphases per person) and 50 metaphases from ten normal 20-year-old human females (five metaphases per person).

Numerical information on the relative position of the chromosomes in metaphases was obtained as previously described.^{5 7 8 10}

Briefly, the co-ordinates of all chromosomes in each metaphase plate were recorded and a circular

transformation was then performed according to Barton *et al*¹¹ to obtain the 'generalised square distance Δ^2 ' (between the centromere of two chromosomes), the 'generalised square distance d^2 ' (between the centromere of a chromosome and the centre of gravity of the metaphase), and the 'generalised angle value' (smallest angle formed between a centromere, the centre of gravity, and another centromere). These values allow a direct comparison between the different metaphases independently of their form and photographic magnification.

Within the investigated populations, d^2 values (or Δ^2 angle values) for a given chromosome combination were ranked in histogram classes with equal intervals. When all d^2 values (or Δ^2 or angle values) from all metaphases of a particular population are compiled on one histogram, the distribution provides a reference histogram for this population.

The statistical significance of distributions obtained in the different populations were analysed by χ^2 tests based on calculations with the above mentioned absolute class frequencies. Graphically, however, these classes were compared as percentage frequencies. Three different types of χ^2 comparisons were performed.

(1) χ^2 tests within the control populations and within the XXY population

The distribution of the d^2 , Δ^2 , or angle values of each chromosome combination in a given population was compared with the reference distribution of its own population. In order to obtain independence between the reference distribution and the distribution for a particular chromosome combination, the absolute class frequencies of the distribution of that particular chromosome combination were subtracted from the absolute class frequencies of the reference distribution. Then, by χ^2 tests, it is possible to decide whether the d^2 , Δ^2 , or angle distribution of a given chromosome combination differs significantly from the reference distribution.

(2) χ^2 tests between the control populations and the XXY populations

(a) The distribution of the d^2 or Δ^2 values of each chromosome combination in the XXY population was compared with the corresponding combination in the XX and XY control populations. This type of χ^2 test allows us to decide for the two groups investigated whether the compared distribution belongs to the same population of numbers.

(b) The first class of the distribution of the chromosome combination in the XXY population was compared with the first class of the distribution of the corresponding chromosome combination in the XX and XY control populations. χ^2 tests were then performed by considering only two classes: the absolute frequencies corresponding to the first class of the distribution (lowest Δ^2 values) and the pooled frequencies of the remaining classes. Thus we have only one degree of freedom in this analysis. This type of test has already been defined by us⁷ as a measure of the association tendency between two chromosomes.

The data were processed on a CDC 6400 computer in the Université Libre de Bruxelles—Vrije Universiteit Brussel mathematical centre.

Results

DISTANCE TO THE METAPHASE PLATE CENTRE (d^2 VALUES)

χ^2 test within the control populations and within the XXY population

Table 1 gives for each homologous chromosome pair in the different populations studied the mean d^2

TABLE 1 $\bar{d}^2$ values and rank (r) values for the control and the patient populations

Chromosome	XY		XX		XXY	
	$\bar{d}^2$	r	$\bar{d}^2$	r	$\bar{d}^2$	r
1	1.871	8	1.925	10	1.745*	1
2	2.168	17	2.229	21	1.931	7
3	2.169	18	2.222	19	1.983	11
4	2.264†	21	2.166	18	2.040	15
5	2.410†	23	2.102	15	1.961	10
6	2.254†	20	2.412*	22	2.124	22
7	2.107	15	1.868	8	2.006	13
8	2.055	13	2.068	13	2.071	16
9	2.277	22	2.069	14	2.013	14
10	2.155	16	2.105	16	2.073	17
11	2.233	19	1.952	11	2.215	24
12	2.094	14	2.228	20	1.942	9
13	1.625†	3	1.821	6	1.929	6
14	1.892	10	1.760	2	1.904	4
15	1.757†	5	1.718	1	1.866	3
16	1.805	6	2.069	14	1.931	7
17	1.917	11	1.906	9	2.116*	20
18	1.819	7	1.816	5	2.126	23
19	1.753	4	1.972	12	2.003	12
20	1.877	9	1.790	4	2.110	19
21	1.486*	1	1.787	3	1.909	5
22	1.500*	2	1.858	7	1.861	2
X	3.045	24	2.158	17	2.079	18
Y	2.000	12			2.122	21

Level of significance (two-tailed) *, $p < 0.005$, †, $0.005 < p < 0.01$, ‡, $0.01 < p < 0.025$.

values, the level of significance for the χ^2 test performed between the distribution of the d^2 values for this particular chromosome pair and the reference distribution, and the rank value. Lowest rank values are given to the most centrally located chromosomes.

From this table it is clear that chromosome pair 6 in both controls is located significantly more peripherally in the metaphase plate, while chromosome pairs 13, 15, 21, and 22 are significantly more central in the XY population. In contrast to these findings, chromosome pair 6 is located more centrally and the acrocentric chromosomes are located more peripherally in the XXY population than in both control populations. Moreover, a significantly central location of chromosome pair 1 and a distribution statistically different from the reference distribution of chromosome pair 17 are observed in the XXY population.

d^2 comparison between the XXY population and the control populations

Contrasting with the significant positive correlation coefficient between the $\bar{d}^2$ values of both control populations ($r=0.6931$, significant at the $p=0.01$ level with $n-2=20$ df), correlation coefficients between the $\bar{d}^2$ values of the XXY population and the $\bar{d}^2$ values of the XX population ($r=0.2370$, $df=20$) or the $\bar{d}^2$ values of the XY population ($r=0.4128$, $df=21$) are low and not significant. Since this study deals with a population of subjects with an abnormal complement of gonosomes, it might be interesting to have a closer look at the specific gonosomal $\bar{d}^2$ values. We therefore performed χ^2 tests between the distribution of the d^2 values of the gonosomes in the XXY population and the distribution of the d^2 values of the corresponding gonosomes in the control population. No significant differences were observed for the centromere-centre distances of the X or Y chromosomes between patient and control populations.

Δ^2 DISTANCES AND ANGLE VALUES BETWEEN HOMOLOGOUS AND NON-HOMOLOGOUS COMBINATIONS

χ^2 tests within the control populations and the patient populations

The comparison of the Δ^2 distributions of each chromosome combination with the Δ^2 reference distribution gives a list of chromosome combinations significantly different from the reference distribution. Similar data are obtained for the angle values. Biological significance cannot be assigned to all statistically significant combinations, but might be inferred for those which showed significant deviations

TABLE 2 $\bar{\Delta}^2$ values and mean angle values for those chromosome distributions which were shown by χ^2 tests to present a distribution of both Δ^2 values and angle values significantly different from their corresponding reference distribution

Chromosome distribution	$\bar{\Delta}^2$ value	p	Mean angle value	p
XX				
13-21	3.214	$p<0.005$	79.71	$0.025>p>0.01$
9-13	3.944	$0.05>p>0.025$	86.72	$0.05>p>0.025$
Reference distribution	4.089		91.56	
4-7	4.431	$0.025>p>0.01$	99.85	$0.025>p>0.01$
13-16	4.603	$0.025>p>0.01$	105.89	$p<0.005$
XY				
21-22	2.924	$p<0.005$	87.62	$0.025>p>0.01$
13-15	3.148	$p<0.005$	83.94	$0.05>p>0.025$
14-21	3.251	$p<0.005$	82.32	$p<0.005$
13-18	3.260	$0.025>p>0.01$	83.22	$p<0.005$
13-14	3.331	$p<0.005$	83.23	$p<0.005$
Reference distribution	4.089		91.15	
3-8	4.597	$0.025>p>0.01$	97.50	$0.05>p>0.025$
5-8	4.733	$p<0.005$	94.67	$0.05>p>0.025$
3-4	4.798	$p<0.005$	95.24	$0.025>p>0.01$
X-3	5.678	$0.01>p>0.005$	91.50	$0.05>p>0.025$
XXY				
21-21	3.520	$0.01>p>0.005$	81.96	$0.05>p>0.025$
1-22	3.593	$0.025>p>0.01$	98.27	$0.05>p>0.025$
13-14	3.670	$0.01>p>0.005$	85.77	$p<0.005$
14-15	3.690	$0.05>p>0.025$	87.49	$0.025>p>0.01$
1-16	3.700	$0.05>p>0.025$	91.32	$0.01>p>0.005$
15-21	3.808	$p<0.005$	85.44	$0.05>p>0.025$
Reference distribution	4.087		91.34	
3-4	4.129	$0.05>p>0.025$	93.57	$0.05>p>0.025$
8-18	4.339	$p<0.005$	90.30	$0.025>p>0.01$
11-13	4.499	$0.025>p>0.01$	94.97	$0.05>p>0.025$

for both Δ^2 values and angular values. We therefore listed (table 2), for each population studied, the chromosome combinations which proved to be significantly different from the reference distribution for both the centromere-centromere distances and the angle values: from left to right are the significant chromosome combinations, the mean Δ^2 value of this particular chromosome combination, the level of significance for the χ^2 test performed between the distribution of the Δ^2 values for this particular chromosome combination and the corresponding reference distribution, the mean angle value, and the level of significance for the χ^2 test performed between the distribution of the angle values for this particular chromosome combination and the corresponding reference distribution.

Attention will be paid more directly to the combinations which present a mean Δ^2 value smaller than that in the reference distribution. The chromosomes involved are those which lie more closely to each other than expected in a random

TABLE 3 $\bar{\Delta}^2$ values in XXY, XX, and XY populations for those gonosome-autosome combinations shown to present significantly different Δ^2 distributions by χ^2 tests comparing each Δ^2 histogram in the XXY population with its corresponding histogram in the XX and/or XY population

Chromosome combination	$\bar{\Delta}^2$ value in XXY	$\bar{\Delta}^2$ value in XX	Level of significance Δ^2 XXY Δ^2 XX	$\bar{\Delta}^2$ value in XY	Level of significance Δ^2 XXY Δ^2 XY
3-X	4.160	4.927	0.005 < p < 0.01	5.678	0.005 < p < 0.01
5-X	4.049	4.720	p < 0.005		
6-X	4.074	4.490	NS	5.506	0.025 < p < 0.05
7-X	4.325			5.367	NS
11-X	4.503	4.095	NS	5.443	NS
12-X	4.098	4.739	0.01 < p < 0.025		
6-Y	4.402			4.608	NS
8-Y	4.026			4.285	NS
11-Y	4.712			4.424	NS
17-Y	4.586			4.027	NS
22-Y	4.186			3.231	0.01 < p < 0.05

distribution. Not too much importance can be given to the number of significant associations, since in an organised structure this number is partly dependent on the number of metaphases examined.

As expected from previous work,⁸ acrocentric chromosomes are often involved in associations in both normal and XXY subjects. Associations involving C heterochromatin rich chromosomes are found more often in the XXY population (1-22, 1-16) than in the controls (13-16). There are some additional associations in the XXY population which are more difficult to explain (3-4, 8-18).

χ^2 tests between the different populations

χ^2 tests were performed between the distribution of the Δ^2 values of the different populations to answer two questions.

- (1) Do there exist some modifications of association between an autosome and a gonosome in patients with an abnormal gonosomal complement? Table 3 summarises the results obtained by comparing the XXY population with both control populations for those distributions which were significantly different from the reference distribution in either patient or control population. In the XXY population it seems that the two X chromosomes lie significantly closer to chromosome pairs 3, 5, and 12 than in the XX population, and significantly closer to chromosome pairs 3 and 6 than in the XY population. However, since the mean Δ^2 value of the reference distribution is about 4.087 one would consider them to be not more associated but rather less dissociated. The combination 22-Y for a similar reason is significantly dissociated as compared with the XY control population.
- (2) Do there exist some modifications of the distribution of the gonosomes in patients

with an abnormal gonosomal complement? χ^2 tests were performed to compare the distributions of Δ^2 values for all gonosomal combinations between the XXY and control populations. Although no significant differences between patient and control populations could be established, on this criterion or by association tendency analysis, it was noticeable that the Δ^2 distances between X and Y are smaller in the XXY (4.114) population than in the XY control population (5.329). The distance between the two X chromosomes is unchanged in the XXY (4.103) population as compared to the XX population (4.027).

Discussion

As mentioned earlier, age,^{12 13} culture conditions,^{12 14} mutagens,¹⁵⁻¹⁷ and viral infection¹⁸ all influence the distribution of human chromosomes. Populations examined, therefore, must be as homogeneous as possible. We must first take into account a possible error owing to age variation since it was not possible to collect enough subjects of the same age. Recent studies (Hens *et al*, in preparation) comparing the distributions of the d^2 values for the different chromosome combinations in a population of 20-year-old normal women with the corresponding distributions of the d^2 values in a population of 40-year-old women (correlation coefficient between the mean d^2 values for the different chromosome combinations in the two populations = 0.855) showed that ageing does not significantly modify the d^2 distance parameter but induces some dissociations of acrocentrics.

A second parameter which has to be considered is the importance of intra- and interpersonal variation. The establishment of a significant difference between two populations depends on

finding greater variation between two populations than between subjects within the populations. However, although detailed analysis is not possible if interpersonal variation is not taken into account, the relatively large number of subjects examined in both populations shows the major characteristic differences between the populations.

The most striking observation from our data is that the presence of an extra chromosome coincides with a disturbance of the theoretically stable centromere-centre distribution pattern of human chromosomes. These data might favour the hypothesis of Mikamo¹ that spindle degeneration as a result of intrafollicular ageing may be a mechanism for non-disjunction in trisomies. Moreover, since centromere-centre distances might be related to replication time,⁵ the biological importance of these findings might be related to the modification of the duration of S phase and cell cycle in patients with abnormal karyotypes.¹⁹⁻²²

Non-disjunction might also result from close associations between chromosomes, as shown in trisomy 21.² Our position analysis method, however, does not clearly indicate such associations for sex chromosomes. Indeed, no XX association was observed in the XXY population. However, to analyse this parameter correctly, metaphases with labelled late replicating chromosome(s) would be more useful. An association between X and Y chromosomes might be supported by our findings since it was observed that the $\bar{z}^2$ value of XY is lower in the XXY population than in the XY population. On the basis of these observations we therefore consider that no clear-cut evidence is given by the method of generalised distances for the induction of gonosomal trisomy XXY by association.

As far as associations between autosomes are concerned, one previous work²³ suggests that the presence of a supernumerary chromosome does not significantly modify the association between human acrocentric chromosomes. These results are almost confirmed by our generalised distances analysis of XXY karyotypes. However, it must be mentioned that the acrocentric chromosomes are located less centrally in the XXY population. Moreover, in the XXY karyotypes, the C heterochromatin rich chromosomes are more often associated than in the control populations.

Preliminary results collected by us separately on 21 XXX, 16 XXYY, and 27 XYY karyotypes confirm the results described here. The centromere-centre disturbance seems even more important. No significant XX or YY associations were observed in patients with a supplementary X or Y chromosome, but a decrease of acrocentric associa-

tions was observed with a parallel increase of associations between C heterochromatin rich chromosomes. However, some particular abnormal heteromorphism of acrocentrics or C heterochromatin regions or both may play an important role in the modified centromere-centromere pattern of the chromosome distributions of the patients. Analysis of patients with an abnormal gonosomal complement by C banding and NOR banding would therefore be useful to clarify the possible role of C heterochromatin in the induction of gonosomal trisomies.

Although the study of chromosome organisation in Klinefelter's syndrome is interesting in itself, one may question how relevant this study can be to the origin of non-disjunction. Indeed, since non-disjunction occurs during meiosis it is evident that the analysis of the arrangement of the chromosomes should preferably be done in the parents of cases. However, for acrocentric chromosomes, different authors have shown not only the inheritance of Ag stainability of nucleolus organiser regions,²⁴⁻²⁶ but also the inheritance of acrocentric association patterns.^{27, 28} We therefore suggest that non-disjunction should be analysed by comparative analysis of chromosome arrangement in parents of numerically abnormal subjects and in their progeny.

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