INTERSATELLITE CONNECTIONS OF HUMAN ACROCENTRIC CHROMOSOMES IN ASSOCIATIONS

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Associations of chromosomes were discovered from the existence of intersatellite connections. Hypotheses regarding their hypergeometric, binomial, Poisson, uniform, and exponential distributions were rejected after testing statistical hypotheses for the quantitative distribution of chromosomes of the D and G groups in associations. Agreement between corresponding discrete points of empirical frequencies and theoretical frequencies calculated by the normal law of distribution is explained by differences in the associative power of chromosomes within the D and G groups. The basic numerical characteristics of the quantitative distribution of acrocentrics in 3- and 4-chromosome associations were calculated.

KEY WORDS: chromosomes; acrocentrics; associations; intersatellite connections; statistical analysis.

In human acrocentric chromosomes a tendency toward the formation of satellite associations is observed [5-7]. The presence of a nucleolar organizer in the short arm of acrocentric chromosomes led to the suggestion that associations are due to their participation in the formation of the nucleolus [8, 9]. The hypothesis of a possible link between associations of acrocentric chromosomes and their nondisjunction during mitotic and meiotic division aroused great interest in associations. The study of associations differing in the group affiliation of the associating chromosomes and in the character of quantitative distribution of D and G chromosomes in them has assumed particular importance. The discovery of the general features of the quantitative distribution of D and G chromosomes in associations can be used later by comparison with the distribution of these chromosomes in associations in patients.

In this investigation the quantitative distribution of D and G chromosomes was studied in associations identified by means of a new criterion: the presence of intersatellite connections detectable by staining based on thermal dissociation of silver.

EXPERIMENTAL METHOD

Karyotypes of lymphocytes in cultures of peripheral blood from 10 healthy blood donors aged 20-30 years were investigated. Specimens were prepared from a 72-h culture by the usual procedure without burning. They were stained with a mixture of 3% neutral formaldehyde and ammoniacal silver at 50-60°C with periodic visual control under the low power of the microscope. The ammoniacal silver was prepared by the following formula: A concentrated aqueous solution of ammonia was added drop by drop to an 80% solution of silver nitrate in 0.1 M Walpole's acetate buffer, pH 3.8, until the solution became completely clear. Using the three-letter code recommended by the addition to the Paris Conference on Standardization in Human Cytology [10], the staining method used should be called SHS (satellite connections – S, detected by heating – H, and by the use of silver – S). A minimum of 50 metaphase plates with a complete set of acrocentric chromosomes was investigated from each donor.

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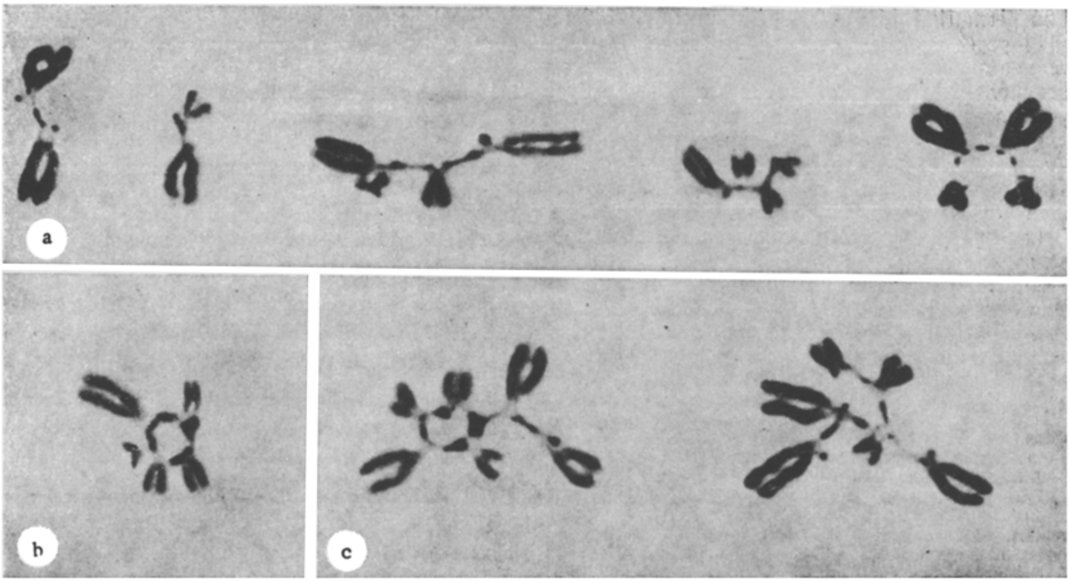

Fig. 1. Variants of associations of human acrocentric chromosomes: a) type of associations most commonly found consisting of a chain formed by successive union of associating chromosomes; b) infrequently found type of associations consisting of a closed ring; c) combined type of association. Stained by thermal silver impregnation (SHS). Objective 90×, ocular 10×.

EXPERIMENTAL RESULTS

The chromosomes were stained yellowish-orange and the satellites brownish-black. Clear fibrillary threads, stained the same color as the satellites, could be seen between the satellites of the acrocentric chromosomes taking part in the association. A group of acrocentric chromosomes whose satellites were joined together by intersatellite connections, irrespective of their mutual orientation and distance apart, was regarded as an association (Fig. 1).

Analysis of 500 metaphase plates revealed 714 associations, which were subdivided into five groups depending on the number of associated chromosomes (from two to six). All possible variants of associations, differing in the quantitative proportions of D and G chromosomes, were counted in each group, but associations of five and six chromosomes were not subjected to statistical analysis because of their small number (eight and three, respectively).

The use of the new staining method and of the criterion of the presence of intersatellite connections for identification of an association requires confirmation of agreement between the empirical quantitative distribution of D and G chromosomes in associations with the existing theoretical laws of distribution.

The quantitative distribution of chromosomes in associations, because of their integrality, was represented as discrete values and the appropriate frequencies. Since the theoretical distribution of discrete random values is characterized by fundamental laws of distribution – hypergeometric, binomial, and Poisson's law – agreement between the empirical data and each of the above laws was tested by Pearson's chi-square criterion. In all cases except associations of four chromosomes in our material, the empirical and theoretical frequencies differed significantly relative to the binomial and hypergeometric laws, i.e., none of the three fundamental laws of distribution of discrete values agreed with the empirical sample in all three groups of associations examined (Table 1).

This can probably be explained on the grounds that the laws of distribution of discrete random values are based on the assumption of equal probability of appearance of the expected event. In the present case, the concept of equal probability embodies a notion of identical, equally possible participation of acrocentrics in associations. Significant differences in empirical and theoretical frequencies, calculated by the laws of distribution of discrete values, in all probability are due to their unequally possible participation in associations of chromosomes forming the D group. Calculations for the quantitative distribution in associations of chromosomes of the G group gave similar results and significant differences between the empirical and theoretical frequencies. Chromosomes in the G group evidently also participate with unequal probability in associations, in agreement with the different frequencies of association for different pairs of acrocentrics [2, 4].

TABLE 1. Testing Agreement between Empirical Data for Quantitative Distribution of D Chromosomes in an Association of 2, 3, and 4 Acrocentrics and Theoretical Values Calculated by Hypergeometric, Binomial, and Poisson Laws of Distribution

Type of association	Empirical frequency n_i	Hypergeometric law			Binomial law			Poisson's law		
		theoretical frequency n_i'	$\frac{(n_i-n_i')^2}{n_i'}$	χ^2 critical	theoretical frequency n_i'	$\frac{(n_i-n_i')^2}{n_i'}$	χ^2 critical	theoretical frequency n_i'	$\frac{(n_i-n_i')^2}{n_i'}$	χ^2 critical
DD	155	176	2,51		190	6,40		142	1,2	
DG	297	282	0,79		253	7,65		218	29,5	
GG	76	70	0,51		85	0,95		168	50,38	
	528	528	3,81	<3,8	528	15,0	6,0	528	81,09	3,8
DD	14	24	4,16		31	9,32		22	2,9	
DDG	68	71	0,13		62	0,58		40	19,6	
DGG	56	43	3,93		41	5,49		50	0,7	
GGG	5	5	0		9	1,78		31	21,8	
	143	143	8,22	3,8	143	17,17	7,8	143	45,0	6,0
DDDD	2	2	0		4	1,0		4	1,0	
DDDG	11	12	0,08		11	0		7	2,3	
DDGG	18	14	1,14		11	4,45		9	9,0	
DGGG	1	4	2,25		5	3,2		8	6,1	
GGGG	0	0	0		1	1,0		4	4,0	
	32	32	3,47	6,0	32	9,65	9,5	32	22,4	7,8

TABLE 2. Testing Agreement between Empirical Values of Quantitative Distribution of D Chromosomes in Associations of 3 and 4 Acrocentrics and Theoretical Values Calculated by Uniform, Normal, and Exponential Laws of Distribution

Type of association	Empirical frequency n_i	Uniform distribution			Normal distribution			Exponential distribution		
		theoretical frequency n_i'	$\frac{(n_i-n_i')^2}{n_i'}$	χ^2 critical	theoretical frequency n_i'	$\frac{(n_i-n_i')^2}{n_i'}$	χ^2 critical	theoretical frequency n_i'	$\frac{(n_i-n_i')^2}{n_i'}$	χ^2 critical
DDD	14	33	10,94		12,7	0,13		15	0,06	
DDG	68	53	4,24		70,6	0,09		26	67,85	
DGG	56	53	0,17		53,6	0,11		20	0,72	
GGG	5	4	0,25		5,6	0,06		52	42,48	
	143	143	15,6	3,8	142,5	0,39	3,8	143	111,11	6,0
DDDD	2	6,5	3,12		1,19	0,55		2,0	0	
DDDG	11	6,7	2,76		13,5	0,46		3,1	20,13	
DDGG	18	6,7	16,07		15,5	0,40		5,1	32,63	
	1	6,7	4,85		1,79	0,35		8,2	6,32	
GGGG	0	5,4	5,4		0,02	0		13,6	13,6	
	32	32	32,2	6,0	32,0	1,76	6,0	32,0	72,68	7,8

Legend. Associations of two chromosomes were not included in the calculation for the number of degrees of freedom for them in the uniform and normal distributions was 0.

During a discrete distribution the total probability of 1 is concentrated in a finite system of points X_i (point distribution of the mass of probability), and in a continuous distribution the mass of probability is represented by a continuous band along the whole of the OX axis or along certain parts with a definite density.

The following model may be used to study the distribution of a discrete value X_i (i = 0.004) in associations of 2, 3, and 4 chromosomes. Let us suppose that the probability P_i is concentrated, not in a discrete system of points X_i, but uniformly distributed over the interval (X_i −0.5; X_i +0.5) with a probability P_i at each point of this interval. In that case we have a continuously distributed random value [3].

Calculations of the theoretical frequencies of appearance of associations of the types examined above, allowing for what has been said, based on the laws of distribution of continuous random values (uniform, normal, exponential) and testing agreement between the empirical and theoretical frequencies by the chi-square criterion reveal that only frequencies distributed by the normal law agree well with the empirical data (Table 2) [7, 8]. In associations of three and four chromosomes we thus have invariance of form (the curve of normal distribution) of the quantitative distribution of chromosomes of the D and G groups.

TABLE 3. Numerical Characteristics of Normal Quantitative Distribution of Chromosomes of D and G Groups in Associations

Type of association	$M(x)$	σG	$V(x)$	As	σAs	Ek	σEk	$Mo(x)$	$Me(x)$
Of three chromosomes	1,64	0,71	43,3%	0,03	0,2	—0,76	0,4	1,64	1,64
Of four chromosomes	2,44	0,66	27,0%	0,55	0,4	—0,05	0,7	2,44	2,44

To assess the quantitative distribution of D and G chromosomes in associations of three and five chromosomes sample characteristics were calculated: mathematical expectation, standard deviation, coefficient of variation, asymmetry, standard deviation of asymmetry, excess, standard deviation of excess, median, and mode (Table 3) [1, 3].

The closeness of the values of the numerical characteristics obtained for associations of three and four chromosomes is evidence that the causal factors lying at the basis of the formation of these associations are common and probably extend also to associations with both more and fewer chromosomes.

Hence, of all the statistical hypotheses put forward, the only one not to be rejected is that of the normal quantitative distribution. Under these circumstances agreement between empirical frequencies and theoretical values calculated by the normal law of distribution, at corresponding discrete points, can be explained by differences in the associative power of the chromosomes within the D and G groups.

LITERATURE CITED

1. V. E. Gmurman, Guide to Problem Solving by the Theory of Probability and Mathematical Statistics [in Russian], Moscow (1975), pp. 274-307.
2. N. S. Zhdanova, "Principles of formation of associations of acrocentric chromosomes in human lymphocytes after treatment with phytohemagglutinin," Author's Abstract of Candidate's Dissertation, Moscow (1975).
3. N. V. Smirnov and I. V. Dunin-Barkovskii, A Course in Probability Theory and Statistics [in Russian], Moscow (1969), pp. 145-152 and 277-278.
4. A. De Capoa, A. Rocchi, and F. Gigliani, Humangenetik, 8, 111 (1973).
5. T. E. Denton, W. M. Howell, and J. V. Barrett, Chromosoma, 55, 81 (1976).
6. M. A. Ferguson-Smith, Lancet, 1, 638 (1961).
7. M. A. Ferguson-Smith, Ann. Hum. Genet., 27, 143 (1963).
8. D. A. Hungerford, in: Mammalian Cytogenetics and Related Problems in Radiobiology (ed. by C. Pavan et al.), Pergamon, New York–London (1964), pp. 133-155.
9. D. G. Harnden, in: Recent Advances in Human Genetics (ed. by L. S. Penrose), Little, Brown, Boston–London (1961), pp. 19-29.
10. "Standardization in human cytogenetics," Cytogenet. Cell. Genet., 15, 203 (1975).