QUANTITATIVE DISTRIBUTION OF SATELLITE ASSOCIATIONS WITH DIFFERENT NUMBERS OF CHROMOSOMES IN HUMAN LYMPHOCYTES

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Statistical analysis of the quantitative distribution of associations formed by 2, 3, 4, 5, and 6 acrocentrics (associations differing in numerical composition) revealed good agreement between the empirical frequencies and the theoretical values calculated by the exponential law of distribution to base 2. The high agreement in all donors tested points to the strict regularity of the quantitative distribution of associations with different numerical values and their special role in human lymphocytes.

KEY WORDS: *satellite associations*; *statistical analysis*; *exponential law of quantitative distribution*.

The discovery of intersatellite connections of acrocentrics participating in satellite associations in man [1] gave a more realistic interpretation of the forces retaining them in the associations and put on a firmer basis the concept of "satellite associations" itself and eliminated subjectivism from their identification. This objectivization of human satellite associations considerably increased the reliability of research and laid the foundations for a more intensive study of their differences, using statistical methods and the theory of probability.

The characteristic features of quantitative distribution of associations of 2, 3, 4, 5, and 6 chromosomes in man are formulated as follows at the present time: with an increase in the number of chromosomes in the composition of associations the number of associations falls significantly. This characterizes the general tendency but does not reflect possible principles governing the distribution of associations with different numbers of associating chromosomes.

In this investigation the principles of quantitative distribution of associations with 2, 3, 4, 5, and 6 chromosomes in healthy donors were studied. Considering the direct role of lymphocytes in the formation of immunological status, disturbance of which is connected with the development of many diseases, human peripheral blood lymphocytes were chosen as the test object.

EXPERIMENTAL METHOD

Karotypes of peripheral blood lymphocytes from 15 healthy donors were investigated. The culture was prepared in the usual way. Specimens were prepared from a 72-h culture by the usual procedure, without flaming.

Satellite association was revealed by the SHS staining method [2]. A group of acrocentric chromosomes connected by intersatellite bonds, irrespective of their mutual orientation or degree of spacing, was taken as an association. Depending on the number of acrocentrics participating, associations with 2, 3, 5, and 6 chromosomes were distinguished. The quantitative distribution of associations formed by 2, 3, 4, 5, and 6 chromosomes was analyzed statistically in two samples, from 10 and 5 donors respectively, and in the combined sample.

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TABLE 1. Probabilities of Appearance of Associations with Different Numbers of Chromosomes (empirical and theoretical, calculated by the law of exponential distribution)

Number of chromosomes in association	Probability	
	empirical	theoretical
2	0,7428	0,750002
3	0,195108	0,187466
4	0,04209	0,046875
5	0,015358	0,011719
6	0,00455	0,002930
7	0,000568	0,000732
8	0	0,000183
9	0	0,000046
10	0	0,000011

EXPERIMENTAL RESULTS

To obtain statistical series, the choice of theoretical distribution curves expressing the principles embodied in them began with the law of exponential distribution, with which a certain similarity was detected a priori. However, comparison of the empirical frequencies by Pearson's criterion with the theoretical frequencies calculated for an exponential distribution to the base of natural logarithms (e = 2.7182) and did not show agreement. Agreement between the empirical frequencies and theoretical calculated for an exponential distribution to base 2 was found by the method of least squares.

Theoretical frequencies of associations containing different numbers of chromosomes form a geometrical progression with denominator 1/4 and with a number of terms equal to the number of possible types of associations, i.e., nine. The mathematical model of the theoretical quantitative distribution of associations with different numbers of chromosomes is expressed by the formula:

$$n_i' = \frac{2^{2(6-i)+1} \sum_{i=2}^{10} n_i}{\sum_{i=2}^{10} 2^{2(6-i)+1}} = \frac{(0.25)^i}{0.08(3)} \sum_{i=2}^{10} n_i,$$

where i is the number of chromosomes in the association; n_i the empirical frequencies; n'_i the theoretical frequencies.

Considering that associations of 7 and 8 chromosomes are extremely rare, and that with 9 and 10 chromosomes are hypothetically possible with very low theoretical probability, the exponential power in the formula is represented by a polynomial containing the number 6. It symbolizes the largest number of chromosomes discovered empirically in the associations. The possibility of formation of associations of more than 6 chromosomes is not ruled out, but for the calculations of theoretical frequencies it is advantageous to use the equation after transformation, in which $(0.25)^i/0.08(3)$ is the theoretical probability of appearance of an association containing i chromosomes ($i = \overline{2.10}$), the values of which are given in Table 1.

High agreement of the empirical frequencies of quantitative distribution of associations of 2, 3, 4, 5, and 6 chromosomes with the theoretical values calculated by the law of exponential distribution to base 2 was found initially in a sample of 714 associations in 10 healthy donors ($\chi^2_{obs} = 1.2$ when $\chi^2_{crit} = 7.8$), and later good agreement with the exponential distribution was obtained in a sample of 1040 associations in five donors, and finally, in the combined sample of 1754 associations from all 15 donors (Table 2).

It is very important to note that the law of quantitative (exponential) distribution of associations of 2, 3, 4, 5, and 6 chromosomes was confirmed in small 50-metaphase samples from all 15 donors. Absence of agreement in the first sample from donor T-in (Table 3) was due to the random increase in the number of 5- and 6-chromosome associations in the sample. In the second 50-chromosome sample from the same preparation good agreement was discovered with the theoretical distribution. In the presence of good agreement of the quantitative

TABLE 2. Comparison by χ^2 of Empirical Quantitative Distribution of Associations of 2, 3, 4, 5, and 6 Chromosomes with Theoretical Values Calculated by Equation of Exponential Distribution (72-h culture of peripheral blood from healthy donors)

Number of chromosomes in associations	500 metaphases			750 metaphases			1250 metaphases		
	n_i	n'_i	χ^2_{obs}	n_i	n'_i	χ^2_{obs}	n_i	n'_i	χ^2_{obs}
2	528	536,02		778	780,76		1306	1316,78	
3	143	134,01		198	195,10		341	329,20	
4	32	33,5		42	48,80		74	82,30	
5	8	8,38		17	12,20		25	20,58	
6	3	2,09		5	3,05		8	5,14	
Total	714	714,00	1,2	1040	1040,00	4,14	1754	1754,00	3,89

distribution in associations of 2 and 3 chromosomes the basic increase in the values of χ^2_{obs} took place mainly on account of large associations consisting of 4 or 5 and, in particular, of 6 chromosomes.

The results of the investigation of three 50-metaphase samples from five donors and the combined data for two and three samples indicate an irregular distribution of large associations in the preparation, and this affects the degree of agreement between the empirical and theoretical frequencies (in donor K-va in the first 50-metaphase sample χ^2_{obs} = 3.32, in the second sample 10.06, and in the third ideal agreement was found between the empirical and theoretical frequencies, χ^2_{obs} = 0.25).

Whereas representativeness of the sample was achieved during the investigation of combined data for several donors during analysis of 50 metaphases from each of them, in the case of individual investigation no fewer than 150 metaphases had to be investigated in order to obtain representativeness.

The degree of agreement between the theoretical probabilities calculated by the suggested law of distribution with the empirical values determines the degree of expression of the principles actually in operation. Good agreement between the theoretical and empirical probabilities confirms the view that the law of exponential distribution expresses the existing principle in the distribution of associations with different numbers of chromosomes in human lymphocytes.

Given that the basic causes of formation are common to all associations, the law of exponential distribution can be used to calculate the theoretical probabilities of infrequent (7 and 8 chromosomes) and hypothetically possible (9 and 10 chromosomes) associations. For instance, by the law of exponential distribution the likelihood of appearance of one association with 7 chromosomes is 1:1365 (P7 = 0.000,732), 8 chromosomes 1:5461 (P8 = 0.000,183), 9 chromosomes 1:21,844 (P9 = 0.000,046) and, finally, 10 chromosomes 1:87,376 (P10 = 0.000,011).

Existing distribution of associations with different numbers of associating chromosomes in the lymphocyte population of human peripheral blood can thus be fully described mathematically. The existing patterns are reflected by the law of exponential distribution, which gives good agreement not only with large total samples, but also with individual samples from each donor. This last fact seems, in the writer's opinion, to be particularly important. Agreement between the quantitative distribution of associations of 2, 3, 4, 5, and 6 chromosomes and the law of exponential distribution in individual samples from donors can be regarded as evidence of the nonrandom formation of associations with different numbers of chromosomes.

This high level of agreement between the empirical data and the law of exponential distribution, first, points to the strict regularity of the quantitative distribution of associations with different numbers of associating chromosomes, second, it is evidence of the special role of the quantitative proportions between associations of 2, 3, 4, 5, and 6 chromosomes in the body, and third, it suggests the existence of definite regulatory mechanisms responsible for maintaining constancy of the appropriate quantitative distribution of associations in the population of human peripheral blood lymphocytes.

TABLE 3. Result of Comparison (by χ^2 method) of Empirical Quantitative Distribution of Associations of Different Numbers of Chromosomes with Theoretical Calculated by Equation for Exponential Distribution in Healthy Donors (72-h culture of peripheral blood, 50-cell samples)

Donor	Sequence	Number of associations	χ^2 observed	χ^2 critical
I-ov	I	74	1,82	
M.	I	53	2,18	
D	I	56	2,41	
K-ка	I	106	3,59	
B-ev	I	94	0,39	
Ch	I	56	3,76	
Zh-ev	I	61	5,24	
K-ov	I	74	5,29	
B-ov	I	63	1,04	
T-in	I	69	50,07	
	II	70	6,8	
K-ot	I	55	2,83	
	II	56	2,79	
	Σ	111	1,56	
	III	71	1,64	
	Σ	182	2,59	
K-va	I	87	3,34	
	II	72	10,06	7,8
	Σ	159	13,97	
	III	85	0,25	
	Σ	244	6,35	
I-yan	I	55	9,84	
	II	51	1,65	
	Σ	106	4,40	
	III	54	1,87	
	Σ	160	3,96	
Kas-va	I	87	10,18	
	II	92	2,4	
	Σ	173	2,32	
	III	74	6,22	
	Σ	247	6,47	
Kr-ov	I	69	4,31	
	II	70	10,58	
	Σ	139	11,3	
	III	71	2,4	
	Σ	244	7,02	7,8

It is impossible at the moment to judge the ways in which this quantitative constancy is regulated. Nevertheless, it seems most likely that the level of regulation is extracellular. In that case regulation could be effected at the level of the organs of lymphopoiesis by the production of lymphocytes with a sufficient number of associating chromosomes to ensure maintenance of the constancy of their quantitative distribution in the population at a certain level.

The discovery of the principles of quantitative distribution of satellite associations was possible, it should be pointed out, only after the development of the method of staining intersatellite connections and the use of the criterion of identification of associations on the basis of the presence of intersatellite connections of chromosomes forming associations.

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