COMPARISON OF TWO METHODS OF IDENTIFYING SATELLITE ASSOCIATIONS IN MAN

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Two methods of identifying satellite associations were compared in ten healthy blood donors. When the conventional Giesma staining method was used, 31% fewer 3-chromosome associations, 50% fewer 4- and 5-chromosome associations, and 66.6% fewer 6-chromosome associations were identified than by staining with ammoniacal silver. Meanwhile, 10% more 2-chromosome associations were identified, on account of the predominance of small associations. Staining with silver when used to detect intersatellite associations revealed acrocentric chromosomes in close juxtaposition and mutually oriented, but with no connections between the satellites. In Giemsa preparations these chromosomes are regarded as associated, with a consequent increase in the number of associations. In the traditional Giemsa staining method neither error is corrected. Identification of associations based on the presence of intersatellite connections after staining with silver is free from this disadvantages.
KEY WORDS: chromosomes; satellite associations; intersatellite connections; identification of associations.

Identification of satellite associations in man is nowadays based on two criteria: the mutual orientation of the short arms of acrocentric chromosomes and the distance between them. It is considered that this distance should not exceed the transverse diameter of the chromosome [2], half the length of the long arm of the largest D-group chromosome in the corresponding metaphase plate [1], or the length of the G chromosome [3]. Variation of the distances between the acrocentric chromosomes makes it more difficult to identify associations and introduce a subjective element into their evaluation, so that errors arise in the determination both of the number of associations and of the number of chromosomes participating in these associations.

Hot silver staining reveals fibrils between the satellites of acrocentric chromosomes taking part in associations; the presence of these fibrils provides an easy method of determining associations and the number of chromosomes participating in them.

The results of identification of associations by the conventional method and by a method based on the presence of intersatellite connections between acrocentrics participating in the associations are compared in this paper.

EXPERIMENTAL METHOD

Metaphase plates from a 72-h culture of peripheral blood from ten healthy blood donors were studied. The blood was cultured in the usual way. Films were prepared without flaming and were stained separately by the Giemsa method and with ammoniacal silver. For Giemsa staining, a solution (1:50) of standard Romanovsky's azure-eosin was made up in distilled water, and the films were stained in it for 15 min (this method of staining will be described conventionally as RV). On examination of films stained by the Giemsa method, acrocentrics mutually oriented with their short arms and lying at a distance apart not greater than half the length of the largest group D chromosome in that metaphase plate were taken as an association.

To detect intersatellite connections a method of staining based on thermal dissociation of silver was used. According to the three-letter code recommended in the appendix to the Paris Conference on Standardization in Human Cytogenetics (1971, 1975), the staining method is described as SHS: Satellite associations, detected by Heat, and with the use of Silver.

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Fig. 1. Associations of acrocentric chromosomes in film from 72-h culture of human peripheral blood lymphocytes. a) Linear association formed by consecutive union of four acrocentrics; D chromosome at end of association lies a considerable distance from the other three acrocentrics, preventing it from inclusion in an association on staining by Giemsa method; b) linear satellite association formed by five acrocentrics; c) association of three acrocentrics all a considerable distance apart; d) stellate associations of 6 chromosomes: 3 group D and 3 group G acrocentrics; e) satellite association of three acrocentrics; the nearby and well oriented group D chromosome is not joined with the associating chromosomes by intersatellite connections and so is not included in the associations; f, g) two acrocentrics well oriented with their short arms and close together, with no intersatellite connections. SHS staining; 900×.

A group of acrocentric chromosomes whose satellites were joined by intersatellite connections were regarded as an association, regardless of their mutual orientation or distance apart. Metaphases with a full set of acrocentrics were included in the analysis. Fifty cells stained by the Giemsa method and the same number of cells stained by the SHS method were analyzed for each of ten individuals.

EXPERIMENTAL RESULTS

Clear fibrils stained the same brownish black color as the satellites were discovered between the satellites of acrocentric chromosomes in associations (Fig. 1). To compare the results of identification of associations by the two methods the following indices were used: a) the total number of associations discovered, associative indices, and the mean number of chromosomes per association; b) the number of metaphase plates without associations, the total number of metaphases containing associations, with their quantitative differentiation by the number of associations (1, 2, 3, and 4) in one metaphase plate.

The results of the comparison are given in Table 1. Great differences were found in the number of associations detected by the two methods. By the conventional method (RV) 16.6% fewer associations (118) were identified in 500 metaphase plates. This means that every sixth association was not confirmed. A definite relationship was found between the size of the associations and the error of their identification: with an increase in the number of chromosomes in the associations, the magnitude of the error increased. For instance, whereas 10% of associations consisting of two chromosomes i.e., every 10th, were not identified, every third of the 3-chromosome association was not identified (31.5%). Every second of the 4- and 5-chromosome associations (53% and 50% respectively) and two of every three 6-chromosome associations (66.6%) were not identified.

TABLE 1. Comparison of Quantitative Parameters Indicative of Associations Identified by the Criterion of Proximity of Acrocentrics and Their Mutual Orientation (RV) and by the Criterion of Possession of Intersatellite Connections (SHS)

Staining method for identifying associations	Number of metaphases	Number of associations	Associative index	Number of chromosomes per association	Number of metaphases: without associations	Number of metaphases: with associations	No. of metaphase plates with undermentioned no. of associations: 1	2	3	4	No. of associations with undermentioned no. of chromosomes: 2	3	4	5	6
SHS	500	714	1,43	2,30	17	429	193	182	50	4	528	143	32	8	3
RV	500	596	1,19	2,24	108	392	214	150	28	0	478	98	15	4	1
Difference: absolute		118	0,24	0,06	37	37	21	32	22	4	50	45	17	4	2
%		16,6					10,9	17,7	44,0		9,5	31,5	53,1	50,0	66,6

TABLE 2. Comparison (by χ^2 criterion) of Frequencies of Associations with Different Numbers of Chromosomes and Frequencies of Metaphase Plates with Different Numbers of Associations According to Results Obtained during Identification of Associations by Two Different Methods

Number of chromosomes in association	Distribution of frequencies after staining by SHS: n_i	SHS: n_i''	RV: n_i'	χ^2: observed	χ^2: critical	Metaphases differing in number of associations	Method of staining SHS: n_i	Method of staining RV: n_i'	χ^2: observed	χ^2: critical
1	2	3	4	5	6	7	8	9	10	11
2	528	440	478			Without assoc.	71	108		
3	143	120	98			with 1	193	214		
4	32	27	15			with 2	182	150		
5	8	7	4			with 3	50	28		
6	3	2	1			with 4	4	0		
	714	596	596	20,81	6,0		500	500	45,71	6,0

Legend. n_i) Empirical frequencies after SHS staining; n_i') empirical frequencies after RV staining; n_i'') frequencies after SHS staining reduced to size of empirical sample after RV staining.

Significant differences were found between the frequencies of 2-, 3-, 4-, 5-, and 6-chromosome associations identified by the two methods compared by means of the χ^2 criterion (Table 2, columns 1-6).

After identification after staining by Giemsa's method the number of metaphase plates without associations was increased by 7.4% and the number of metaphases with associations was reduced correspondingly. The distribution of the latter was shifted toward metaphases with a single association. Every second metaphase with three associations and every sixth metaphase with two associations were unidentified, and only the number of metaphases with one association was approximately 10% higher, because of small 2-chromosome associations.

No clear quantitative relationship was observed between the decrease in the number of metaphase plates with 2, 3, and 4 associations and the increase in the number of metaphases containing 1 association. The significant differences according to Pearson's criterion (Table 2, columns 7-11) obtained by comparing the frequencies of metaphase with different numbers of associations revealed by the two different staining methods, prove that they were not random in nature.

In the writers' opinion, the increase in the number of metaphases with one 2-chromosome association on identification after staining by the Giemsa method is attributable to differences in the spatial arrangement of the acrocentric chromosomes in large associations. The mutual arrangement of acrocentrices, especially in linear associations formed by consecutive union in the form of a broken line, does not always correspond to the criteria used to identify associations after staining by conventional methods. This is particularly true of large linear associations consisting of 4, 5, 6, and 7 chromosomes. The mutual orientation of the short arms of acrocentrics is often absent in such associations and, what is particularly important, the permitted dis-

tances between them are considerably exceeded. On Giemsa staining such a combination could not be identified as an association. The detection of intersatellite connections between acrocentrics not mutually oriented and relatively distant from one another, when the SHS staining method is used, served as the basis for identification of such groups as associations (Fig. 1c). Sometimes in large associations a distinctive grouping of acrocentrics within an association was revealed by SHS staining: they were well oriented in groups of 2 or 3 and lay close together, whereas 2 acrocentrics from different groups were joined by an intersatellite connection, uniting these groups into a single association. On staining by the Giemsa method the independent groups of acrocentrics under these conditions were identified as separate small associations and not as a single large association. As a result, the number of small associations was increased at the expense of one large association.

In conclusion, another special feature with a substantial effect on the character of distribution of associations when identified on the basis of proximity and mutual orientation of chromosomes must be emphasized. On SHS staining, 58 acrocentrics well oriented with their short arms with other acrocentrics, and at a distance apart such that they could be regarded as constituents of associations, were discovered in 2-, 3-, and 4-chromosome associations. However, they were not joined by intersatellite connections with the adjacent acrocentrics, and consequently they were not included in associations. When associations were identified on the basis of mutual orientation and proximity of acrocentrices, all these 58 chromosomes without exception were included in associations; besides increasing the total number of associating chromosomes, this also increased the number of large associations quite unjustifiably.

The quantitative distribution of frequencies of associations identified by the conventional method is thus influenced by two mutually opposite tendencies. One, due to the character of grouping of acrocentrics in large associations, causes a shift toward an increase in the number of smaller associations, whereas the other, due to the inclusion of chromosomes unconnected by intersatellite connections in associations, causes a shift in the opposite direction toward an increase in the number of large associations. The factors responsible for these shifts cannot be allowed for quantitatively, and for that reason the results of investigations by the conventional method cannot be appropriately corrected.

LITERATURE CITED

1. N. S. Zhdanova, "Principles governing the formation of associations of acrocentric chromosomes in human lymphocytes after treatment with phytohemagglutinin," Author's Abstract of Candidate's Dissertation, Moscow (1975), p. 5.
2. A. A. Prokof'eva-Bel'govskaya, V. M. Gindilis, K. N. Grinberg, et al., Tsitologiya, No. 2, 158 (1966).
3. K. B. Zang and E. Back, Cytogenetics, 7, 455 (1968).