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Quantitative Analysis of C-bands in Chromosomes 1, 9, 16, and Y of Twins

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Thirty-two pairs of Caucasoid twins, 16 monozygotic (MZ) and 16 dizygotic (DZ) of the same sex, were studied by densitometry in relation to the C-bands of chromosomes 1, 9, 16, and Y. Confirming earlier results, concordance was not absolute among MZ. Estimates of the degree of genetic determination for these traits varied from 0.73 to 0.89 for the autosomes and from 0.86 to 0.95 for the Y. There are now stronger indications that a fraction of the intergeneration variability found in these structures may be real, probably due to mitotic and/or meiotic unequal crossing-over.

Key words: C-bands, Heterochromatin, Chromosome variability, Densitometric analysis of chromosomes

INTRODUCTION

The factors that may affect the transmission of the constitutive heterochromatin from one generation to the next are still poorly understood. This is due in part to the small number of families or twin series studied [2,3,5,10,11,16,18] and also to the technical problems that interfere in the C-band measurements (review in [6]). In a previous twin study [19], we verified that the degree of genetic determination for these traits was less than the expected 100%. Since this investigation was performed with a relatively crude method of C-band determination, we decided to reinvestigate the same sample using a more refined, microdensitometric evaluation.

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MATERIALS AND METHODS

A total of 32 twin pairs were studied, 16 monozygotic (MZ) and 16 dizygotic (DZ) of the same sex. All were healthy Caucasoids, with ages varying from 18 to 48 years; their socioeconomic level could be described as medium or high. The C-bands were obtained by Sumner's [17] technique. Further details about the sample and the methods employed can be found in the report of Viégas and Salzano [19]. For the present investigation, the C-bands, plus the euchromatic regions of the long arm of chromosome 1 and of the Y, were measured directly from the photographic negatives in a Zeiss microscope equipped for densitometric analysis (MPM 01 photometer head Servogor RE 541 recorder). Three metaphase plates from each individual were studied. Further description of the technique employed is given by Erdtmann et al [7–9]. Since the C-band limits were determined visually directly under the microscope, ten cells (seven from male persons) were chosen to obtain an evaluation about the amount to intraobserver variation in duplicate measurements from the same cell. In a total of 67 comparisons, the average difference found was 0.3% of the structures measured.

All C-band measurements were corrected for the variable stage of chromosome contraction using as a reference the euchromatic region of the long arm of chromosome 1. The relationship between the rate of contraction in the eu- and heterochromatin varies with the size of the region considered [8]; therefore, for the indicated correction it is necessary to apply the formula presented by Erdtmann et al [7], introducing the average value and the slope of the regression between the eu- and heterochromatin sizes obtained in the present sample. The resulting expression is the following:

$$H_c = H + \left[(2.84 - E) \frac{0.273H - 0.094}{1 + 0.273\ (2.84 - E)} \right]$$

where H_c = C-band size corrected for chromosome contraction; H = the C-band measurement, and E = the size of the euchromatic region lq-h. The quantity (2.84 − E) represents the difference between the stage of contraction of the cell that is being considered and the general average for all cells (2.84 μm). The expression (0.273H − 0.094) establishes the slope of the regression of each band relative to the euchromatic variation, but this slope is only applicable to bands that are in an average level of contraction. Therefore, it is necessary to apply a correction, dividing the last expression by [1 + 0.273 (2.84 − E)].

RESULTS AND DISCUSSION

The results obtained are shown in the Table. The averages found for the C-band absolute sizes were somewhat lower than those encountered in other samples from Moscow, Hradec Králové, and Porto Alegre [1,7,9,14]. The relative sizes, however, were quite close to those obtained in these studies. The averages of the indices of heteromorphism were also very similar to those previously observed [9,12]. We can therefore conclude that the twins studied here seem to represent an unbiased sample of the population from which they have been drawn.

Estimates of the degree of genetic determination (h^2) considering the C-band absolute sizes are presented in the Table. The autosome values vary from 0.73–0.89. Viégas and Salzano [19], using the same sample studied here and the same formula, but a simpler method of C-band measurements, obtained 0.64, 0.73, and 0.40 for chromosomes 1, 9, and 16, respectively. It is clear, therefore, that the employment of a more refined technique significantly increased these estimates, making them also interchromosomally more consistent. The use of relative sizes lead to further increases in three of the four estimates, confirming the view of Podugolnikova and Korostelev [12] and Podugolnikova et al [13] that since sizes are less susceptible to intercell variation they should be preferred in studies of twin zygosity.

The Y distal C-band gives h^2 values (0.86–0.95) closer to that expected theoretically (1.00). However, if the total size of this chromosome is considered, the estimate is

*TABLE I. Average C-band Sizes, Indices of Heteromorphism, and Estimates of the Degree of Genetic Determination of These Characteristics in a Sample of 16 MZ and 16 Same-Sex DZ Twins**

Characteristics	Chromosomes			
	1	9	16	Y
Absolute size (μm) (average ± SD)	1.06 ± 0.22	0.89 ± 0.15	0.69 ± 0.14	0.90 ± 0.15
No. of observations[a]	96	96	96	21
Relative sizes (%)[b]	40	34	26	—
Indices of heteromorphism (h^-/h^+; average ± SD)[c]	0.80 ± 0.10	0.81 ± 0.11	0.84 ± 0.09	—
No. of observations	64	64	64	—
Degree of genetic determination[d]				
Absolute size	0.73	0.75	0.89	0.86[e]
Relative size	0.80	0.78	0.83	0.95
Indices of heteromorphism	0.33	0.56	0.33	—

*Note that the degree of genetic determination for the total heterochromatic regions of these chromosomes was estimated as 0.66.

[a]Only one member of each MZ pair was considered in this calculation.

[b]Relative to the total of heterochromatin of chromosomes 1, 9, and 16.

[c]h^-, Smaller C-band; h^+, larger C-band; SD, standard deviation.

[d]According to Clark's [4] formula.

[e]The value considering the total size of Y was 0.78.

lowered (0.78). This suggests the occurrence of important variability in its euchromatic portion. The total amount of constitutive heterochromatin of the four chromosomes, as well as their indices of heteromorphism, varied little between MZ and DZ, conditioning low h^2 numbers (0.33–0.66) for these characteristics.

The values for the degree of genetic determination of C-band sizes presented here may still be underestimates; but there are now stronger indications that a fraction of the intergeneration variability obtained may be real, being due to mitotic and/or meiotic unequal crossing-over. Further insight on this problem could be obtained using high resolution techniques (like those employed by Schempp and Müller [15]), as well as larger twin samples, considered together with those of their parents.

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