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CHROMOSOME SURFACE AREA

Further Evidence for Autosomal Sexual Dimorphism

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SUMMARY

The karyotypes of 76 males and 84 females, each assembled by the trypsin banding method, are examined in a study designed to investigate sex differences among autosomes. It is shown that female autosomes have consistently larger surface areas than the males, with respect to both the short and long arm measurements. In addition, discriminant function analysis is used to distinguish between the male and female karyotypes. We find that, using autosomal measurements alone, this can be done with a high probability of success.

In a recent paper we showed that female autosomes were consistently longer than male autosomes [5]. The karyotypes of 100 males and 100 females, each assembled by the trypsin banding method, were used in this and the average lengths of each of the autosomes were computed separately for each sex. We found the average value for the female cells to be consistently longer than that of the male cells, and that this was mirrored in both the short and long arms. With the single exception of the short arm of chromosome number 19, the arms of the female autosomes were longer than the corresponding arms of the male autosomes. Analysis of the arm ratios and centromere indices of these chromosomes also revealed certain shape differences, but the pattern of these differences proved to be more difficult to characterize. Discriminant function analysis was also used to differentiate between male and female cells. Depending on which set of measurements was used in the discriminant function analysis, some 75% of the cells could be correctly classified as either male or female on the basis of autosomal measurements alone.

The purpose of the present paper is to report analogous results obtained on the basis of surface area measurements. It is shown that female autosomes have consistently larger surface areas than the male autosomes. Certain "shape" differences also exist, when these are defined in the obvious way using arm ratios and centromere indices. In addition, the technique of discriminant function analysis is used to classify karyotypes as either male or female. Using only autosomal surface area measurements, we again find that this identifica-

tion can be made with a relatively high probability of success.

METHODS AND MATERIALS

The present study is based on cells cultured from the peripheral blood of 76 males and 84 females. Some of this material is identical with that used in the study alluded to earlier [5]; however, many of the karyotypes which were entirely suitable for measuring arm lengths were less appropriate for the measurement of surface area. When this occurred, we tried to obtain other cells from the original donors whose chromosomes were at an earlier stage of mitosis and hence more readily lent themselves to surface area measurements. This did not prove possible in every instance, accounting for the differences in sample sizes in the two studies. In any event, each of the individuals included were normal, healthy people between 20 and 40 years of age; most of them were employed at the Veterans Administration Hospital in Ann Arbor, Michigan. The cultures were processed following the technique of Moorhead et al. [6]. The slight modifications introduced by us to this method have been documented elsewhere [7]. The chromosomes were stained with Giemsa and the banding patterns were obtained using the "trypsin method" of Seabright [9].

Photomicrographs were taken by an oil-immersion lens with a magnification of 90 diameters and an eyepiece magnification of 10 diameters. These negatives produced prints with a final magnification of approx. 8000 diameters. After photography, the best cell metaphases were selected for readability, absence of overlapping and comparability of the attained stage of colcemid mitosis. Individual chromosomes were cut out from each print and mounted on a card with the homologous chromosome pairs numbered and grouped following the scheme recommended by the Paris Conference [8]. The male and female cells were processed at random and a single batch of colcemid was used to harvest all of the cells.

The surface areas of the chromosomes were measured by recording the coordinates of a number of points located on their perimeters. The procedure was adapted from one developed for the analysis of craniofacial morphology [10], but the principles underlying the technique are immediately applicable in the present context. The points were digitized and their coordinates automatically punched on cards for subsequent computer processing.

The areas of the short (M_1) and long arms (M_2) of each of the autosomes were computed from the coordinate point values by standard computer routines designed for this purpose. From these, we computed the total area of the chromosome ($M_3=M_1+M_2$); the ratio of the short arm to the long arm ($M_4=M_1/M_2$); the centromere index ($M_5=M_1/M_3$); and the relative areas of the short arm, the long arm and the total chromosome which represent these areas as a percentage of the sum of the areas of the total chromosomal complement, excluding the sex chromosomes. These measurements are entirely analogous to those used previously by us [3, 5] simply substituting area for length.

RESULTS

Table 1 gives the sex-species mean values of the measurements defined above for each of the 22 autosomes. The corresponding values for the sex chromosomes are also given, but this is for the sake of completeness only—none of the measurements on the sex chromosomes are used in any of the subsequent analyses designed to identify male/female differences.

Concentrating first on the (absolute) total area of the autosomes, it is seen that the average values for the female cells are consistently larger than those of the male cells. And this is mirrored in both the short and long arm: With the sole exceptions of the short arm of chromosome number 19, the long arm of chromosome number 21 and the total area of chromosome number 21, the arms of the female autosomes are larger than the corresponding arms for the male autosomes.

Differences in chromosomal shape as measured by the areal analogues of the arm ratio and centromere index are also apparent. While there is no obvious, consistent pattern of differences which can be used to distinguish between male and female cells, there are some definite indications of differences within certain of the chromosomal groups. For example, in the D and G groups, the arm ratios are considerably higher for the females than for the males. This is seen also in the context of the centromere indices of these chromosomes.

When one considers the relative measures, however, no evidence for sexual dimorphism emerges. This is, of course, to be expected in the context size differences. The very use of relative measurements cancels out size differences by design [5].

We turn now to the results of the discriminant function analyses which have as

Table 1. *Sex-specific mean values for eight measurements made on each of the 22 autosomes for 76 male and 84 female cells*

Chromosome no.	Sex	Direct measurements			Ratios		Rel. measurements		
		Short arm	Long arm	Total area	Arm ratio	Centromere index	Short arm	Long arm	Total area
1	♂	83.36	87.31	170.67	0.955	48.8	4.43	4.65	9.08
	♀	91.10	99.90	191.00	0.923	47.8	4.44	4.86	9.30
2	♂	61.36	97.73	159.09	0.628	38.5	3.26	5.21	8.47
	♀	69.10	107.04	176.14	0.647	39.2	3.36	5.21	8.57
3	♂	61.80	71.84	133.64	0.866	46.3	3.29	3.81	7.10
	♀	69.89	76.47	146.36	0.917	47.7	3.40	3.72	7.12
4	♂	32.50	89.94	122.44	0.363	26.5	1.72	4.78	6.50
	♀	35.82	99.46	135.28	0.361	26.4	1.74	4.85	6.59
5	♂	31.45	86.33	117.78	0.367	26.8	1.66	4.57	6.23
	♀	34.31	95.35	129.66	0.362	26.5	1.67	4.63	6.30
6	♂	43.34	74.00	117.34	0.586	36.9	2.30	3.94	6.24
	♀	47.34	81.80	129.14	0.579	36.6	2.30	3.99	6.29
7	♂	39.30	65.85	105.15	0.599	37.4	2.09	3.51	5.60
	♀	43.78	71.49	115.27	0.615	38.0	2.13	3.48	5.61
8	♂	31.95	63.11	95.06	0.505	33.4	1.69	3.36	5.05
	♀	33.36	68.42	101.78	0.491	32.7	1.62	3.34	4.96
9	♂	32.36	57.51	89.87	0.570	36.0	1.72	3.06	4.78
	♀	36.96	59.94	96.90	0.627	38.3	1.80	2.91	4.71
10	♂	28.32	60.98	89.30	0.468	31.7	1.51	3.24	4.75
	♀	31.57	66.12	97.69	0.485	32.5	1.54	3.21	4.75
11	♂	31.48	55.62	87.10	0.567	36.0	1.67	2.95	4.62
	♀	33.91	62.14	96.05	0.551	35.4	1.65	3.02	4.67
12	♂	24.61	61.69	86.30	0.405	28.7	1.32	3.28	4.60
	♀	28.01	66.38	94.39	0.430	29.7	1.37	3.23	4.60
13	♂	8.46	59.82	68.28	0.147	12.6	0.462	3.19	3.65
	♀	10.97	66.64	77.61	0.168	14.2	0.538	3.24	3.78
14	♂	7.76	56.58	64.34	0.141	12.2	0.421	3.02	3.44
	♀	9.42	60.56	69.98	0.158	13.5	0.463	2.95	3.41
15	♂	7.78	54.00	61.78	0.144	12.4	0.406	2.87	3.28
	♀	9.84	60.32	70.16	0.166	14.1	0.484	2.94	3.42
16	♂	24.06	37.28	61.34	0.645	39.1	1.27	1.98	3.25
	♀	24.24	40.47	64.71	0.603	37.4	1.18	1.96	3.14
17	♂	16.60	39.46	56.06	0.425	29.6	0.881	2.09	2.97
	♀	17.13	40.07	57.20	0.433	29.8	0.831	1.94	2.77
18	♂	13.41	38.05	51.46	0.353	25.9	0.709	2.02	2.73
	♀	14.40	42.32	56.72	0.341	25.3	0.700	2.06	2.76
19	♂	18.67	24.56	43.23	0.769	43.2	1.00	1.31	2.31
	♀	18.50	25.32	43.82	0.737	41.9	0.891	1.23	2.12
20	♂	18.30	24.70	43.00	0.749	42.6	0.973	1.32	2.29
	♀	19.57	25.84	45.41	0.773	43.3	0.953	1.25	2.20
21	♂	6.43	22.64	29.07	0.286	22.0	0.343	1.20	1.54
	♀	6.60	21.76	28.36	0.310	23.3	0.323	1.05	1.37
22	♂	6.17	22.67	28.84	0.276	21.4	0.328	1.20	1.53
	♀	8.29	23.24	31.53	0.377	26.8	0.405	1.12	1.52
X	♂	39.65	62.07	101.72	0.642	38.9	2.10	3.29	5.39
	♀	40.36	68.71	109.07	0.589	37.0	1.97	3.35	5.32
Y	♂	7.30	28.02	35.32	0.268	20.8	0.387	1.48	1.87

Table 2. *Results of the discriminant function analysis when the direct measurements of the short arms of the chromosomes are used as the set of discriminating variables*
Numbers in parentheses are percentages

	Male	Female	Total
Male	63 (82.9)	13 (17.1)	76
Female	18 (21.4)	66 (78.6)	84

Mahalanobis distance	*F*-statistic	*P*-value
2.68	4.22	0.0001

their aim the estimation of the extent to which male and female cells can be distinguished on the basis of autosomal measurements. In addition, tests of the significance of the differences in the mean values for the males and females are provided. The use of this technique in a cytogenetic context was illustrated by Harris et al. [3] and Kowalski et al. [5]. A more general discussion was given by Kowalski [4]. Table 2 gives the results when the direct short arm measurements were used as the set of discriminating variables. It is seen that 63 (82.9%) of the 76 male cells were correctly classified as male, while 13 (17.1%) were misclassified as being female. Similarly, 66 (78.6%) of the 84 female cells were correctly classified and 18 (21.4%) misclassified. The Mahalanobis distance (sometimes called the D^2-statistic [1]) is a measure of the distance between the mean values of the distributions of these variables in the male and female samples and, in the two-group case, is simply related to Hotelling's T^2-statistic which is used to test the hypothesis that the mean values of the set of discriminating variables are the same for the males and females [4]. The more general (in the sense that is can be used with more than two groups) *F*-statistics is shown in the table along with the associated *P*-value.

When different sets of variables are used in subsequent discriminant function analyses, the Mahalanobis distance may be used to compare the discriminating power of these different sets of variables; the larger the distance, the more the mean values of the variables are separated in the two groups. The *P*-value may be used to test the significance of this separation and the classification matrix provides an estimate of the accuracy with which individual chromosome sets can be correctly allocated to the male and female groups.

When the direct measurements of the long arms of the autosomes are used as the set of discriminating variables, the results are as shown in table 3. The total areas of the autosomes are considered in table 4. It is seen that the short arms are as effective as the total areas in terms of classificatory accuracy and even slightly more effective in terms of the Mahalanobis distance.

That shape differences can also be used to distinguish between male and female cells is shown in tables 5 and 6 where the results for the arm ratios and centromere indices are summarized. Both these sets of

Table 3. *Results of the discriminant function analysis when the direct measurements of the long arms of the chromosomes are used as the set of discriminating variables*
Numbers in parentheses are percentages

	Male	Female	Total
Male	63 (82.9)	13 (17.1)	76
Female	21 (25.0)	63 (75.0)	84

Mahalanobis distance	*F*-statistic	*P*-value
2.60	4.09	0.0001

Table 4. *Results of the discriminant function analysis when the direct measurements of the total areas of the chromosomes are used as the set of discriminating variables*

Numbers in parentheses are percentages

	Male	Female	Total
Male	63 (82.9)	13 (17.1)	76
Female	18 (21.4)	66 (78.6)	84

Mahalanobis distance	*F*-statistic	*P*-value
2.55	4.01	0.0001

variables are effective discriminators and, in fact, they fare almost as well as the areas of the arms and the total chromosome areas. The reason for this is that discriminant function analysis consists of finding linear combinations of the set of discriminating variables which maximally separate the groups under consideration. Thus more weight is given to the ratio measurements in the D and G groups resulting in an effective subset of discriminating variables. While the differences in area are consistent, many of the individual differences are small and so the fact that the ratio measurements are essentially as effective as the size measurements is perhaps not too surprising.

Table 5. *Results of the discriminant function analysis when the direct measurements of the arm ratios of the chromosomes are used as the set of discriminating variables*

Numbers in parentheses are percentages

	Male	Female	Total
Male	60 (78.9)	16 (21.1)	76
Female	20 (23.8)	64 (76.2)	84

Mahalanobis distance	*F*-statistic	*P*-value
2.12	3.33	0.0001

Table 6. *Results of the discriminant function analysis when the direct measurements of the centromere indices of the chromosomes are used as the set of discriminating variables*

Numbers in parentheses are percentages

	Male	Female	Total
Male	62 (81.6)	14 (18.4)	76
Female	20 (23.8)	64 (76.2)	84

Mahalanobis distance	*F*-statistic	*P*-value
2.33	3.66	0.0001

We should also mention here that each of the discriminant analyses referred to above was repeated in a stepwise manner in order to rank the variables in order of their discriminatory power and to see whether a subset of the variables would suffice to accomplish the required classification. When this was done for the arm ratio measurements, e.g., we found that the arm ratios of the chromosomes numbered 22, 3 and 9, in that order, were sufficient to effectively discriminate between the sexes. Using only these three measurements in the discriminant functions, 81.6% of the males and 67.9% of the females were correctly classified. This compares quite favorably with the 78.9% and 76.2% obtained previously using the arm ratios of all the autosomes and suggests that, for many purposes, the number of measurements taken can be limited to just these three. Given that these three arm ratios are included in the discriminant functions, the remaining arm ratio values are essentially redundant (by virtue of the correlations between them and the variables already selected) and they may be excluded from the discrimination process. Similar results were obtained for the other

sets of discriminatory variables considered. While the particular autosomes selected differed in some instances, it proved to be true in every case that but a few of the measurements were able to effectively discriminate between the sexes. Thus it would appear that substantial size/shape differences exist between male and female autosomes.

DISCUSSION

The results provide further evidence for autosomal sexual dimorphism in human chromosomes. Not only are the female autosomes longer than the males [5], they are also of significantly greater surface area. Significant differences in shape, as reflected in arm ratios and centromere indices, were also found to exist.

We should perhaps note here that we view the evidence presented in table 1 as the more convincing for the existence of autosomal sexual dimorphism. While the discriminant function analyses provide additional relevant information, and formal tests of hypotheses concerning the equality of the mean values in the two groups, the consistent size differences stand out as clear indicators of sexual dimorphism. No complicated mathematical tools are necessary to establish this fact [4]. Indeed, if classification is the ultimate aim, one should consider the use of recently developed forms of discriminant function analysis which incorporate the homologous pair structure of karyotypes into their formulation [2]. By ignoring this structure our results are conservative in the sense that at least this level of discrimination is possible given the data. Imposing more structure on the procedure can only improve the accuracy with which the karyotypes are classified. In any event, additional evidence for the existence of autosomal sexual dimorphism has been presented and we again point to the lack of explanations for the observed differences [5].

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