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Acrocentric Chromosome Associations in Man

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INTRODUCTION

The five pairs of human acrocentric chromosomes are of interest for at least four reasons: (1) the short arms are involved in associations at metaphase [1]; (2) they show heteromorphism for all three short arm regions (the short arms proper, the satellite stalks, and the satellites themselves) [2]; (3) the short arms are the location for the genes coding for 18S and 28S ribosomal RNA, and the human is highly polymorphic with respect to the number of such genes [3, 4]; and (4) the proximal regions of the acrocentric chromosomes are the sites of breakage and exchange in the formation of Robertsonian translocations, the most frequent structural rearrangement in man [5].

It is often assumed, and it is indeed tempting to speculate, that these four features are causally related, but in spite of a great deal of investigation, there is remarkably little agreement on the existence or nature of the functional interrelationship between any of the above properties of human acrocentric chromosomes. Indeed much of the evidence is conflicting. Thus it has been variously claimed that the probability of a chromosome being in an association is random [6], positively correlated with the size of the satellite stalk [7], positively correlated with the size of the satellites themselves [8], or negatively correlated with the length of the short arm [8].

It has been suggested that there is a heritable difference in the number of sites coding for ribosomal RNA on the chromosome and that this difference is correlated with the length of the satellite stalk [4, 9]. Furthermore, Dittes et al. [9] claimed that the frequency with which an acrocentric chromosome is found in association is proportional to the length of the satellite stalk and thus to the number of ribosomal RNA cistrons. On the other hand, Henderson et al. [3] and Evans et al. [4] have shown by in situ hybridization that chromosomes in association do not hybridize more tritiated ribosomal RNA than those not in association, suggesting that the probability of a chromosome being involved in association is not a simple function of the number of genes it carries which code for ribosomal RNA.

Patil and Lubs [10], in a study of association frequency of acrocentric chromo-

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somes, found that the chromosomes most commonly involved in Robertsonian translocations (i.e., 14, 21, and 13) were also most frequently involved in association. This is consistent with the suggestion of Ohno et al. [11] that acrocentric associations might be related to Robertsonian translocations.

Present evidence suggests that the probability of any chromosome entering into association is a unique and probably heritable feature of that particular chromosome [12, 7]. However, the possible correlations between the likelihood of a chromosome entering an association and other features of the acrocentric chromosome are as yet unresolved. Much of the apparent confusion in attempting to correlate various physical and functional attributes of acrocentric chromosomes seems to us to have resulted from: (1) failure to define the sources of variation in the character being measured; (2) a sample size inadequate to allow for sources of variation; and (3) in the case of associations, the lack of a mathematical theory for the analysis of the trait.

It is the purpose of the present paper to define the sources of variation in the frequency and types of acrocentric association, to present a mathematical theory for their analysis, and to correlate the status of the heteromorphisms of the acrocentric chromosomes with their association frequency. The same material has been used to compare the relative frequency of association of a particular chromosome pair with the amount of ribosomal DNA it contains; the results of these analyses will be reported later (Warburton et al., in preparation).

MATERIALS AND METHODS

Blood was cultured from five Caucasian laboratory personnel (two males and three females) on three separate occasions and from another male on two separate occasions. Cultures were set up in the standard way using Gibco prepared medium 1A and harvested after a 72 hr incubation—the last hour in the presence of colcemid. They were treated for 8 min in hypotonic potassium chloride and fixed in three changes of Carnoy's fixative (3 parts methanol:1 part glacial acetic acid); fixative containing cells was dropped onto clean, dry slides and allowed to dry at room temperature. The slides were G banded using a slight modification of the technique of Gallimore and Richardson [13].

Duplicate cultures were set up from each blood sample, and duplicate slides were made from each culture. The slides were coded and scored blind by two observers. One observer scored the top half of all even numbered slides and the bottom half of all odd numbered slides and vice versa. Each observer scored 10 cells on each slide for associations; therefore, 40 cells were scored from each culture and 80 cells from each blood sample, with the exception of one sample in which one culture was accidentally lost. No distinctive heteromorphic features visible on G-banded chromosomes were present in any individual scored and, with the exception of the sex of the donor revealed by the presence or absence of a Y chromosome, the observers had no clue as to the identity of the cells being scored. Cells were suitable for scoring if all 10 acrocentric chromosomes could be identified. Two or more chromosomes were considered in association if the distance between the distal ends of the short arms and the point at which their long axis intercepted was equal to or less than the length of a G group chromosome. All associations were recorded and subsequently transferred to magnetic tape for computer analysis. After the association scoring was completed, slides from each individual were stained with Atebrin and examined under fluorescent light. The heteromorphic band at the centromere of chromosome 13 and the satellite stalk and satellite of all acrocentric chromosomes were subjectively evaluated by two observers.

SOURCES OF VARIATION IN ASSOCIATION FREQUENCY

The number of cells scored on the six individuals was not equal because subject T41 was not available for the third culture and one culture from subject T45 was lost. Therefore the analysis of sources of variation in the frequency (p) with which different chromosomes were involved in association was done by multiple regression, weighting each estimate of association frequency by the number of associated chromosomes per cell (N). Each associated chromosome was counted once, irrespective of the type of association in which it was involved.

TABLE 1

ANALYSIS OF VARIANCE

Source	df	Mean Squares × 10^5					Pooled p	No. Chromosomes Associated/ Cell
		p_{13}	p_{14}	p_{15}	p_{21}	p_{22}		
Subject (S)	5	1,754	8,853	5,629	8,660	2,074	5,394	7.83
Culture (C)	7	227	309	619	599	785	508	8.99
Observer (O)	1	286	326	1,452	460	239	553	28.81
Slide within S, C, and O	118	391	357	453	450	434	417	2.96

NOTE.—Only the variance among subjects is significant ($P < .001$).

The results of the analysis are shown in table 1. It can be seen that neither the culture, observer, nor the slide is a significant source of variation in the pooled association frequency p. However, there is highly significant heterogeneity among subjects for all five chromosome pairs. The number of associating chromosomes per cell, as opposed to the relative frequency with which each of the five different pairs enters into association, is very variable. All sources of variation are significant in an unweighted analysis but those contributed by different observers are especially striking. This may well be largely due to the selection of different types of cells by different observers rather than any difference in what they score as an association. Thus, the total number of associating chromosomes is obviously an unreliable measure of biological variation, whereas the relative frequency with which different chromosomes enter into association in different individuals is a repeatable and reliable measure of variation among acrocentric chromosomes.

The relative frequency of association of acrocentric chromosomes in the six individuals scored by us is shown in table 2. The results show the combined frequency of association for both homologues. Overall, the chromosome 21 pair is the most frequently associated, followed by the chromosome 22 pair. However, there is wide variation among individuals, and this variation affects all five chromosome pairs. Thus, subject T40 has an excess number of associations involving chromosome 14, while chromosome 21 associations are excessive in subject T42; subject T43 has a deficiency of associations involving chromosome 14, whereas in subject T45, those involving chromosome 15 are few.

TABLE 2

FREQUENCIES OF SATELLITE ASSOCIATIONS

SUBJECT	AGE	SEX	CHROMOSOMES ASSOCIATED*					NO. CELLS SCORED	NO. CHROMOSOMES ASSOCIATED	MEAN NO. ASSOCIATIONS/CELL
			13	14	15	21	22			
T40	46	M	0.196	0.314	0.190	0.129	0.172	240	583	2.43
T41	39	M	0.200	0.157	0.120	0.284	0.241	160	370	2.31
T42	50	M	0.123	0.184	0.142	0.307	0.244	240	661	2.75
T43	37	F	0.178	0.114	0.233	0.260	0.215	240	634	2.64
T44	40	F	0.159	0.168	0.239	0.242	0.191	240	648	2.70
T45	41	F	0.172	0.186	0.116	0.271	0.255	200	517	2.58
Total	...	...	0.168	0.188	0.179	0.250	0.217	1,320	3,413	2.59

* Expressed as proportion of all chromosomes associated in that individual.

The individuals studied by us had no obvious heteromorphisms of the acrocentric chromosomes visible on G banding, enabling the repeated observations to be done completely blind. However, homologues which can be separately identified would be very valuable in testing randomness of association between homologues and in determining genetic control of association frequencies. Ongoing studies of association frequencies within families will help to resolve this problem. Clearly it is essential, in studies of satellite association, that individuals and wherever possible, homologues, be distinguished and their variability allowed for in any test of differences between samples (e.g., between controls and the parents of trisomics). If the two groups are scored blindly, an analysis of variance which takes account of the variation between individuals is acceptable, whereas a χ^2 test which assumes only binomial variation is suspect.

PAIRWISE SATELLITE ASSOCIATIONS

We wish to know not only the frequency with which a given acrocentric enters into association, but also whether the chromosomes with which it associates are random. For this we need a theory for the expected frequencies, which has so far been lacking. Cohen and Shaw [14] wrote: "Ideally these values should be theoretically determined, without resorting to the empiric data; however, such a derivation would demand knowledge of all possible permutations of the acrocentric associations and the frequencies of each type. An obviously monumental undertaking!"

The problem can be made manageable by partitioning an association of n chromosomes into all $n(n-1)/2$ pairs. Then the number of possible outcomes is reduced and the derivation of expectations is simplified. Consider a set of N acrocentrics, where $N = 10$ for a diploid, $5k$ for a k-ploid, and 8 for a Robertsonian translocation. Let p_i be the frequency of the ith type among associated chromosomes ($i = 1, \ldots, 5$). Assuming that p_i for an individual is constant between homologues and among cultures and observers and that association is random, the probability under sampling without replacement that a pair of associated chromosomes include types i and j is

$$P_{ij} = \begin{cases} \dfrac{p_i\,(p_i - 1/N)}{1 - 1/N} & \text{for } i = j \\ \dfrac{2\,p_i\,p_j}{1 - 1/N} & \text{for } i \neq j. \end{cases} \tag{1}$$

If all acrocentrics enter equally into association, then in a k-ploid $p_i = 2/10$; for a trisomic $p_i = 3/11$; and for the eight chromosomes remaining when there is a Robertsonian translocation, $p_i = 2/8$. Values of p_i significantly different from these expectations are evidence either that chromosomes enter unequally into association or that they dissociate unequally during preparation of the slide. The likelihood ratio test is used to compare observed and expected frequencies [15].

Wright [16] showed that if the correlation F between associated pairs is constant, the association probabilities may be written as

$$P_{ij} = \begin{cases} p_i^2 + p_i(1 - p_i)F & \text{for } i = j \\ 2p_i p_j(1 - F) & \text{for } i \neq j. \end{cases} \tag{2}$$

Making this substitution in equation 1 we have

$$1 - F = \frac{N}{N - 1} \tag{3}$$

which gives $F = -1/9$ for five pairs of acrocentrics. Now we relax the assumption that p_i is constant between homologous, within groups, and among individuals, relatives, slides, and observers, but still assuming that F is constant. Then

$$1 - F = (1 - F_W)\ (1 - F_A) \left(\frac{N}{N - 1} \right). \tag{4}$$

Here F_W is a negative fraction due to heterogeneity between chromosomes within an individual; F_A is a positive fraction due to nonrandom association or heterogeneity among individuals, cultures, slides, or observers; and $-1/(N - 1)$ is the correlation between pairs drawn without replacement from a set of N. Tests of hypotheses developed for factor-union phenotype system are directly applicable to equation (4) [15]. If heterogeneity in F between homologous pairs of chromosomes is significant, the generalized Wright's model which contains an F parameter for each pair of chromosomes is appropriate [17].

These formulas are easily extended to multiple associations, but it is more convenient to partition n-tuple associations into $n(n - 1)/2$ pairs. Estimates of parameters are unbiased, but the error variance is inflated because such pairs are not independent. If a χ^2 test is used then lack of significance may be accepted, but an apparently significant result should be confirmed by analysis of variance.

Table 3 gives the distribution of pairwise associations for our six subjects and the pooled data of Mattei et al. [18], the only comparable observations on banded material known to us. In our data the null hypothesis ($p_0 = 1/5$, $F_0 = -1/9$) is clearly excluded, but there is a good fit to equation (2) when p_i and F are estimated simultaneously ($\chi^2_{54} = 61.5$). The estimate of F varies significantly among individuals ($\chi^2_5 = 11.4$). Since F is a random variable dependent on genotype, a sufficiently large sample would show significant variation among chromosomes. If the p_i and F are estimated separately for each individual and the estimates pooled over individuals, the weighted mean for our subjects is $F = -.124 \pm .007$. This deviation from $-1/9$ is in the predicted direction, since F_W in equation (4) is negative.

In the data of Mattei et al. [18] there is an apparently significant excess of 21–22 pairs and a deficiency of 14–22 pairs; however this has not been confirmed by analysis of variance. It is possible that there are differences between individuals

TABLE 3

DISTRIBUTION OF SATELLITE ASSOCIATIONS BY CHROMOSOME PAIRS ($p_0 = 1/5, F_0 = -1/9$)

SUBJECT	HOMOLOGOUS PAIRS					HETEROLOGOUS PAIRS										χ^2 TESTS			
	13–13	14–14	15–15	21–21	22–22	13–14	13–15	14–15	13–21	13–22	14–21	14–22	15–21	15–22	21–22	p_0,F_0	$\hat{p},F_0$	$p_0,\hat{F}$	$\hat{p},\hat{F}$
T40	6	20	6	2	1	54	32	50	16	28	38	53	19	27	14	93.8	12.3	92.9	10.1
T41	5	1	2	11	10	15	16	11	30	18	22	22	18	7	36	50.1	11.2	49.4	11.0
T42	2	2	3	30	15	24	19	30	37	20	55	45	42	35	93	147.7	13.8	147.6	13.1
T43	7	2	7	15	5	14	41	20	44	32	24	26	69	46	60	90.3	18.4	87.8	10.0
T44	5	5	10	17	8	23	32	46	40	24	41	31	49	46	39	31.6	6.0	31.5	5.9
T45	3	6	0	23	11	32	11	21	42	41	29	38	26	25	55	86.0	13.3	85.8	11.4
Total Hawaii	28	36	28	98	50	162	151	178	209	163	209	215	223	186	297	...	...	...	...
Mattei et al. [19]	179	150	175	288	221	734	734	693	873	741	807	643	800	712	1,009	...	...	...	...
Total χ^2	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	499.6	75.0	495.1	61.5
df	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	84	60	78	54

NOTE.—In our data only the deviation from the null hypothesis of equal association frequencies is clearly significant ($P < .001$).

both in the relative frequency with which certain chromosomes are found in association and in the chromosomes with which they pair. The possibility of nonrandom association should be tested in other samples.

Several studies of pairwise satellite associations have been made in nonbanded material by contrasting the D and G groups [1, 19–22]. These data are not significantly heterogeneous, the failure of variations in techniques of cell culture, preparation, and scoring to affect the estimates being impressive. To some extent, of course, agreement is exaggerated by the loss of power to detect variation among individuals in nonbanded material. The pooled estimates are $p = .5663 \pm .0024$ and $F = -.0736 \pm .0071$. The greater tendency of G group chromosomes to associate is indicated by an estimate of p significantly less than .6. Taking the strength of D group association as unity, the strength of G group association is $(.6/.4)\ (.4337/.5663) = 1.149$. Clearly F does not agree with its theoretical value of $-1/9$. Most of this deviation is due to heterogeneity of the p_i within the D and G groups, which does not contribute to F when homologous pairs are distinguished. The advantages of using banded material are obvious.

CORRELATION BETWEEN ASSOCIATION FREQUENCY AND CHROMOSOME PHENOTYPE

An attempt was made to see whether there was any correlation between the phenotype of the acrocentric chromosomes with respect to their heteromorphic regions and the frequency with which they were associated. Observations on quinacrine banded chromosomes are given in table 4. While these observations are subjective, they are the consensus of two observers, and we feel that they are reasonably accurate with respect to the more outstanding heteromorphic features. Because of the claims made by a number of investigators of a positive correlation between the length of band p12 and the amount of association and because it has been suggested that band p12 is the site of the cistrons coding for 18S and 28S ribosomal RNA, we paid particular attention to this heteromorphism. However, in our material there seems to be no obvious correlation between any heteromorphism recorded and the degree of satellite association. For example subject T45 has obvious satellite stalks and satellites on both no. 22 chromosomes which are frequently found in association in this individual being second only to the chromosome 21 pair. On the other hand, subject T43 has obvious satellites and satellite stalks on both no. 13 chromosomes and yet they are not very frequently found in association. The lack of correlation between the degree of association and the phenotype of the chromosomes seems conclusive in the six individuals studied by us. However, they were selected because they had no obvious heteromorphisms visible on G banding. Thus it is still possible, although it seems unlikely, that extreme forms of certain heteromorphisms of independent origin are consistently correlated with frequency of association.

SUMMARY

Heterogeneity among chromosomes was found to be a highly significant source of variation for association proportions, while culture, slide, and observer were negligible sources of variation for association proportions although important for

TABLE 4

QUINACRINE OBSERVATIONS ON ACROCENTRIC CHROMOSOMES

SUBJECT	CHROMOSOMES AND BANDS										
	13			14		15		21		22	
	cen	p12	p13	p12	p13	p12	p13	p12	p13	p12	p13
T40	35	4	44	3	44	2	33	3	33	2	00
	35	2	00	2	23	1	44	1	22	2	43
T41	35	2	00	4	45	3	45	3	23	3	45
	0	2	00	2	33	1	44	2	00	2	12
T42	25	2	00	2	00	3	22	3	33	2	33
	00	2	00	3	33	2	44	2	00	2	22
T43	00	3	55	3	44	3	45	3	34	1	43
	00	2	33	1	33	1	13	3	34	3	33
T44	35	2	00	2	55	1	34	3	34	1	43
	35	1	23	3	33	2	00	4	33	2	00
T45	35	2	00	3	33	0	44	3	44	3	43
	35	2	00	3	33	2	00	2	20	3	43

NOTE.—All heteromorphic regions were scored on a scale of 0–5 for size (0 = absent, 1 = very small, 2 = small, 3 = medium, 4 = large, 5 = very large) and the centromere region of 13 and the satellites were scored on a scale of 1–5 for brightness [2]. In bands cen and p13 where both size and brightness were evaluated the results are recorded as a double digit, the first number being the size and the second the brightness, whereas in band p12 only the size was recorded which appears as a single digit.

numbers of associations. The consequences of these results for tests of group differences are discussed.

It seems evident that each pair of acrocentric chromosomes has its own characteristic probability of entering into association. This is presumably a combination of the probability for each individual member of the pair, a proposition easily tested utilizing acrocentric chromosomes carrying polymorphisms which allow each member of the pair to be individually recognized.

A mathematical theory for pairwise satellite association was developed and shown to fit observations on banded chromosomes. While we found very significant heterogeneity among individuals in the frequency with which different chromosomes entered into associations, there was no significant evidence for preferential association between any particular chromosomes, either heterologous or homologous. This finding in our material of apparently random associations between different chromosomes is contrary to claims made by other investigators and should be tested on other material. No correlation was found between the phenotype of the chromosome, as judged by cytogenetic polymorphisms, and its probability of association.

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