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Satellite DNA Sequences in the Human Acrocentric Chromosomes: Information from Translocations and Heteromorphisms

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SUMMARY

Satellite III DNA has been located by in situ hybridization in chromosomes 1, 3–5, 7, 9, 10, 13–18, 20–22, and Y and ribosomal DNA (rDNA) in the acrocentric chromosomes 13–15, 21, and 22. In the acrocentric chromosomes, the satellite DNA is located in the short arm.

Here we report comparisons by in situ hybridization of the amount of satellite DNA in Robertsonian translocation and "normal variant" chromosomes with that in their homologs. In almost all dicentric Robertsonian translocations, the amount of satellite DNA is less than that in the normal homologs, but it is rarely completely absent, indicating that satellite DNA is located between the centromere and the nucleolus organizer region (NOR) and that the breakpoints are within the satellite DNA.

The amount of satellite DNA shows a range of variation in "normal" chromosomes, and this is still more extreme in "normal variant" chromosomes, those with large short arms (p+ or ph+) generally having more satellite DNA than those with small short arms (p− or ph−). The cytological satellites are heterogeneous in DNA content; some contain satellite DNA, others apparently do not, and the satellite DNA content is not related to the size or intensity of fluorescence of the satellites. The significance of these variations for the putative functions of satellite DNA is discussed.

INTRODUCTION

The human acrocentric chromosomes (D group: chromosomes 13, 14, and 15, and G group: chromosomes 21 and 22) have been the subject of extensive investigation.

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Two of them are involved in the only viable autosomal trisomies (trisomy 13, Patau, and trisomy 21, Down syndrome) and are thus of particular clinical significance, and all of them may be involved in Robertsonian translocations. These are the commonest form of chromosome rearrangement in man, although the frequencies with which specific chromosomes are involved in such rearrangements differ [1].

Robertsonian translocations are of two kinds, differing in the sites of the breakpoints that give rise to the translocation. If the breakpoints are in the short arms of both chromosomes, a dicentric chromosome is formed, together with an acentric fragment that is lost. If one breakpoint is in a short arm and the other in a long arm, a monocentric chromosome is formed, together with a small chromosome consisting of one centromere with its original short arm and the distal region of the short arm of the other chromosome. This small reciprocal product also seems to be lost, although the bi-satellited marker chromosomes found in some individuals may have arisen in this way. Most of these bi-satellited marker chromosomes, however, appear to be derived from chromosome 15 [2], while the majority of Robertsonian chromosomes are derived from chromosomes 13 and 14; this argues against such an origin, as does the absence of Robertsonian translocations in most individuals with these marker chromosomes.

The short arms of the acrocentric chromosomes show a range of polymorphisms. The secondary constrictions, which contain the ribosomal RNA (rRNA) genes [3, 4], vary in length and in the amount of rDNA present. The short arms also contain satellite DNAs, and study of Robertsonian translocations suggests that these are located between the centromere and the NOR [1, 5]. Finally, the acrocentric chromosomes are highly polymorphic for the presence or absence, size, and intensity of fluorescence of cytological satellites, the composition of which, in terms of DNA sequence, is unclear.

Here we describe the effect on satellite DNA content of losses of chromosome material as a result of translocations, and of variation in size and fluorescence of each of the short-arm regions.

MATERIALS AND METHODS

Individuals with chromosomal translocations and variants were identified in surveys or selected from the karyotype register maintained in this unit; requests for peripheral blood samples were made through the individual's family doctor. The karyotypes of these individuals are given in table 1. Chromosomes were prepared from leukocytes by the method of Evans et al. [6], and the dicentric or monocentric nature of the Robertsonian translocations identified by kinetochore staining essentially as described by Denton et al. [7] with the substitution of 1 hr in 2 × SSC at 60°C for the pretreatment with NaOH, and the use of a uv strip light instead of a photoflood (G. Spowart, personal communication, 1980).

Satellite III DNA was prepared from total placental DNA in 32-ml preparative Ag^+-Cs_2SO_4 gradients. First-cycle gradients contained DNA at 70 μg/ml, an Ag^+/DNA-P ratio of 0.2, and a final density judged by measurement of the refractive index of 1.48 g/cm^3. The gradients were centrifuged to equilibrium in the Sorvall OTD65 using the TV850 titanium vertical rotor at 40,000 rpm. Equilibrium of these vertical gradients was achieved in 20 hrs. Fractions of 0.5 ml were collected from the top of the gradient by injecting Fluorinert FC-48 (M.S.E., Crawley, Sussex, England) into the bottom of the tube via a peristaltic pump and a

TABLE 1

KARYOTYPES OF INDIVIDUALS IN THIS STUDY

Case	Karyotype	Origin
A	45,XX,−13,−14,+tdic(13,14)	Familial
B	45,XY,−13,−14,+tdic(13,14)	"
C	45,XX,−13,−14,+tdic(13,14)	De novo, maternal origin
D	45,XX,−13,−14,−tdic(13,14),22s+	Familial
E	68,XXY,−13,−14,+t(13,14)	Triploid spontaneous abortus de novo
F	45,XY,−13,−14,+t(13,14)	Familial
G	45,XY,−13,−22,+tdic(13,22)	"
H	45,XX,−14,−15,+tdic(14,15)22s+	"
J	45,XY,−14,−15,+tdic(14,15)	"
K	45,XX,−14,−21,+tdic(14,21)	"
L	45,XX,−14,−21,+tdic(14,21)	"
M	45,XX,−21,−22,+t(21,22)	"
N	45,XX,−21,−22,+t(21,22)	"
O	46,XX,13p−	"
P	46,XX,13p−	"
Q	46,XX,13p−,22p+	"
R	46,XY,15p−,22p+	"
S	46,XY,13p−,22p+	"
T	46,XY,9qh+,13ph+,s+	"
U	46,XX,13ph+,s+	"
V	46,XY,14p+	"
W	46,XX,15p+	"
X	46,XX,15ph+,s+	"
Y	46,XY,21ph+,22s+	"
Z	46,XX,22s+	"

NOTE: None of these cases is related to any other, except for J and K, and M and N (see text).

21-gauge needle. The A_{260} was measured with a Pye Unicam SP1750 spectrophotometer. Satellite I was essentially pure after this step, and a new satellite, L_2, formed a separate peak between satellite I and the main band. L_2 has a buoyant density in CsCl of 1.699 g/cm^3 and is distinct from satellite III although related to it (A. J. Clark, in preparation). In conventional angle rotors, L_2 is not separated from satellite III, and is isolated with it. Satellite III itself was a shoulder on the light side of the main band, and pooled fractions in this shoulder were adjusted to 1.44 g/cm^3 in a final volume of 32 ml, centrifuged for 20 hrs, and fractionated as above. A clear shoulder or separate peak on the light side was obtained, and the fractions composing it were pooled and dialyzed exhaustively against 5.0 M NaCl to remove the silver. Following this, the DNA was pelleted by overnight centrifugation in the MSE 10 × 10 Ti rotor at 45,000 rpm in the MSE Superspeed 65 or Prepspin 65. The pellets were dissolved in 0.01 M Tris-HCl, pH 7.8, and finally purified in preparative 4.2 ml CsCl gradients (density of 1.70 g/cm^3) in the TV865 high-speed titanium vertical rotor of the Sorvall OTD65 at 55,000 rpm for 20 hrs. Fractions of 0.1 ml were collected, and the tubes containing satellite DNA were pooled, diluted with 0.01 M Tris-HCl, pH 7.5, pelleted, and resuspended in 0.01 M Tris-HCl, pH 7.5, and stored frozen at −20°C. Purity and identity of satellite III were confirmed by analytical ultracentrifugation as described previously [8], and restriction endonuclease digestion with Hae III and agarose gel electrophoresis [9]. Satellite III digested with Hae III gives a characteristic multimeric series based on a monomer of 170 base pairs (bp).

Preparation of complementary RNA (cRNA) from ^{3}H-labeled precursors and in situ hybridization were as described previously [8, 10, 11]. Preparation of rRNA and in situ hybridization were as described by Gosden et al. [1].

RESULTS AND DISCUSSION

The results were analyzed quantitatively as described [10]. This method of analysis gives extremely consistent results and emphasizes the quantitative nature of in situ hybridization described by Szabo et al. [12].

The data in table 2(A) show that in dicentric Robertsonian translocations some satellite DNA can always be detected in association with each centromere, although the quantity of satellite is almost always less than that associated with the normal homolog's centromere. In monocentric translocations, on the other hand, no satellite DNA can be detected in the chromosome arm that has lost its centromere,

TABLE 2

HYBRIDIZATION OF SATELLITE III cRNA TO THE ACROCENTRIC CHROMOSOMES

CASE		CHROMOSOME NO. 13	14	15	21	22
	A. Robertsonian translocations					
A	Average grains*	n† 1.63	1.13	3.19	0.35	1.35
		t† 0.75	0.75	...	...	...
	% difference‡	−53	−34	...	...	...
B	Average grains	n 0.28	1.16	0.7	0.2	0.4
		t 0.24	0.61	...	...	...
	% difference	−14	−42	...	...	...
C	Average grains	n 0.65	1.53	1.34	0.84	1.03
		t 0.33	0.33	...	...	...
	% difference	−43	−78	...	...	...
D	Average grains	n 2.06	2.2	4.36	1.1 n	2.13
		t 0.26	0.53	...	... ++†	3.73
	% difference	−80	−76	...	...	+75
E	Average grains	n 0.33	1.02	1.17	0.22	0.47
		t 0.13	0	...	...	...
	% difference	−60	−100	...	...	...
F	Average grains	n 1.18	0.89	1.37	0.29	0.57
		t 0.12	0.01	...	...	...
	% difference	−90	−99	...	...	...
G	Average grains	n 0.65	0.96	0.84	0.03 n	0.9
		t 0.15	...	...	... t	0.78
	% difference	−77	...	...	...	−13
H	Average grains	3.0 n	4.29	4.86	3.68 n	4.64
		... t	1.36	1.71	... +	6.07
	% difference	...	−75	−71	...	+34
J	Average grains	0.48 n	1.19	1.29	0.52	0.89
		... t	0.26	0.15	...	...
	% difference	...	−78	−88	...	...
K	Average grains	2.26 n	2.0	2.17 n	1.79	2.46
		... t	0.9	... t	1.09	...
	% difference	...	−78	...	−51	...
L	Average grains	0.09 n	0.61	1.01 n	0.5	1.0
		... t	0.48	... t	0.29	...
	% difference	...	−21	...	−42	...
M	Average grains	0.3	1.0	1.9 n	0.4 n	1.0
		...	...	... t	0.4 t	0
	% difference	...	...	...	0	−100
N	Average grains	0.11	0.57	1.48 n	0.75 n	0.58
		...	...	... t	0.35 t	0
	% difference	...	...	...	−53	−100

TABLE 2 (continued)

CASE		CHROMOSOME NO. 13		14		15		21		22
				B. Short-arm variants						
O	Average grains.....	n	1.02		0.99		2.05		0.84	1.28
		−†	0.02		...		...		...	...
	% difference		−98		...		...		...	...
P	Average grains.....	n	0.60		0.54		0.95		0.35	0.98
		−	0		...		...		...	...
	% difference		−100		...		...		...	...
Q	Average grains.....	n	0.46		0.81		1.68		0.62	n 0.29
		−	0.07		...		...		...	+ 1.34
	% difference		−85		...		...		...	+362
R	Average grains.....		0.65		0.75	n	0.95		0.55	n 0.45
			...		...	−	0.15		...	+ 0.77
	% difference		...		...		−84		...	+71
S	Average grains.....	n	0.61		0.63		0.88		0.65	n 0.44
		−	0		...		...		...	+ 0.94
	% difference		−100		...		...		...	+114
T	Average grains.....	n	2.65		2.75		4.45		2.55	3.9
		+	3.65		...		...		...	...
	% difference		+38		...		...		...	...
U	Average grains.....	n	10.4		11.6		15.0		8.3	11.5
		+	13.1		...		...		...	...
	% difference		+27		...		...		...	...
V	Average grains.....		0.2	n	0.23		0.57		0.19	0.47
			...	+	1.33		...		...	...
	% difference		...		+478		...		...	...
W	Average grains.....		0.99		0.97	n	1.54		0.85	1.04
			...		...	+	1.42		...	...
	% difference		...		...		−6		...	...
X	Average grains.....		5.38		4.13	n	13.5		6.23	6.81
			...		...	+	15.8		...	...
	% difference		...		...		+17		...	...
Y	Average grains.....		2.85		4.0		5.2	n	3.1	n 2.55
			...		...		...	+	5.4	+ 2.55
	% difference		...		...		...		+62	+32
Z	Average grains.....		6.05		5.4		7.15		5.05	n 5.5
			...		...		...		...	+ 6.65
	% difference		...		...		...		...	+18

* Average grains: the average no. grains per chromosome in at least 20 cells, less background.

† n: The normal homolog of a heteromorphic chromosome pair; t: the homolog involved in a translocation. +: a variant with an increase in short-arm material or fluorescence (see table 1). —: a variant with a decrease in short-arm material (see table 1).

‡ % Difference: the difference between the normal and translocation or variant chromosomes, as a percentage of the no. grains on the normal chromosome.

where the centromere can be identified by quinacrine fluorescence (see E, F, M, and N). In addition to the intrinsic errors of the experiment, the actual amounts of satellite DNA detected are subject to two factors leading to variation: the variation that exists between individuals, and the different amounts of chromosome short-arm material lost in different translocations. Only in cases such as C, in which the parental chromosomes from which the translocation was derived are available for study, can the first of these sources of variation be evaluated and the difference be precisely related to loss of short-arm material.

Table 2(B) shows the differences in amount of satellite DNA found between normal chromosomes and those with structural variants. Here again, it is not possible to determine an absolute value for the variation between normal chromosomes. However, some of the variants appear to have little or no effect on the amount of satellite DNA. For example, case W shows a 15s+ with fractionally *less* satellite DNA than its homolog; cases X and Z show less than a 20% difference between the variant and normal homologs. The question of the satellite DNA content of cytological satellites will be discussed later, but in the cases described above, the differences may not be associated with the presence of the variant, but due simply to variation between karyotypically normal chromosomes.

Dicentric translocations can thus occur with very small losses of satellite DNA-containing material. However, we found that both dicentric and monocentric Robertsonian translocation chromosomes contained less satellite DNA than their normal homologs. Chromosome 21 of case M (table 2) was an exception. Cases M and N are cousins, and the translocation chromosome t(21,22) is the same in both, although the normal homologs are derived from different parents. The nearly identical values for hybridization to the translocation chromosome in the two individuals supports the validity of our numerical analysis. From this, we can say that the normal chromosome 21 in N has more satellite DNA than its counterpart in M.

The breakpoints in dicentric Robertsonian translocations must almost always be within the region containing satellite DNA, as this DNA is consistently reduced in quantity, but rarely completely lost. Brasch and Smyth [13], Mattei et al. [14], and Mikkelsen et al. [15] have examined Robertsonian translocations, using silver staining to detect NORs. In a total of 54 cases, only two showed silver-positive regions indicating the presence of an active NOR. That rDNA was present but inactive can be confirmed only by hybridization of an rRNA or rDNA probe in situ. Our investigation of the 12 Robertsonian translocations listed in table 1 detected no rDNA, confirming that in general the breakpoints are within the satellite DNA region. Mikkelsen et al. [15] found the reciprocal product of a t(13,15) monocentric translocation; this small chromosome was silver-positive, although the large t(13,15) was not, confirming that the translocation resulted in the deletion of rDNA. In the case of the monocentric translocations (E and F, as well as M and N), one chromosome arm has no satellite DNA detectable by in situ hybridization. Where it is possible to identify the remaining centromere, it is always that of the chromosome that still retains detectable satellite DNA, supporting our findings [1, 5] that all the satellite DNA in the acrocentric chromosomes is located in the short arm.

The distribution of satellite DNA *within* the short arm is shown by the variants of the acrocentric chromosomes, in particular by those which have an extended NOR as well as distinct cytological satellites (as in cases T, U, V, and W). In these, the separation of the cytological satellites from the centromere gives clear resolution of the location of autoradiographic grains, as hybridization to the cytological satellites is distinguishable from hybridization to the short arm.

The cytological satellites of different individuals obviously have considerable differences in their DNA composition. Figure 1*a* and *c* show clear, heavy, autoradi-

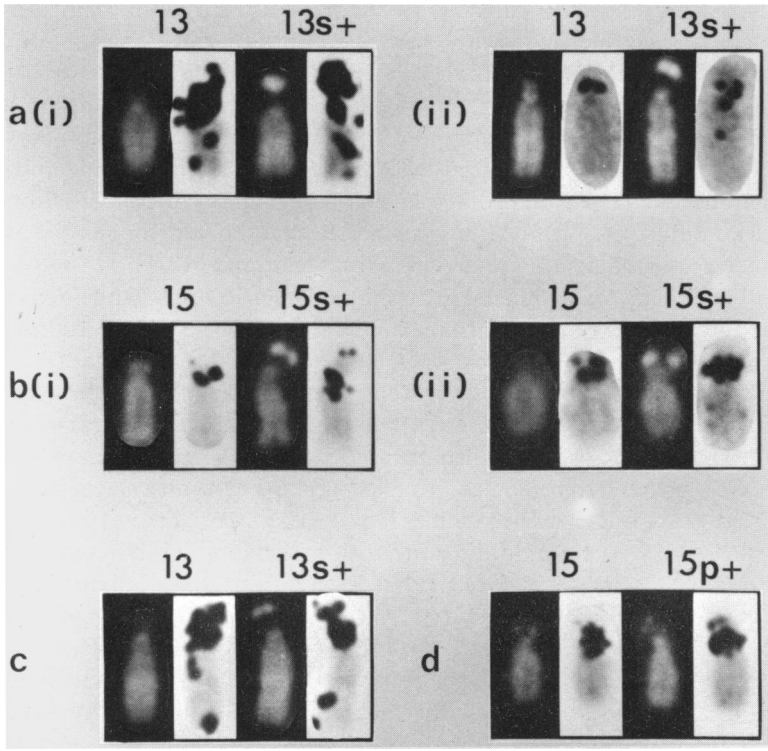

FIG. 1.—Hybridization in situ of satellite III cRNA and ribosomal RNA. In each case the quinacrine fluorescence photograph is followed by the autoradiograph of the same chromosome. *a*, Case U (46XX,13ph+s+) hybridized (*i*) with satellite III cRNA and (*ii*) with ribosomal RNA; *b*, case X (46XX,15ph+s+) hybridized (*i*) with satellite III cRNA and (*ii*) with ribosomal RNA; *c*, case T (46XY,9qh+,13ph+s+) hybridized with satellite III cRNA; *d*, case W (46XX,15p+) hybridized with satellite III cRNA.

ographic labeling of the cytological satellites when hybridized with cRNA to satellite DNA III, while *b* has very little label and *d*, none at all. Figure 1*a* and *c* are both 13ph+s+, while *b* is 15ph+s+ and *d* is 15p+. We have too few cases providing sufficiently clear results to be sure if this distinction between the DNA content of cytological satellites on chromosomes 13 and 15 is universal.

Figure 1*a* and *b* also show the hybridization of these chromosomes with rRNA. The site of hybridization of the rRNA is the secondary constriction (as Evans et al. [3] showed), so that in the 13ph+s+ in 1*a*, the rDNA is located between two blocks of satellite DNA (in the short arm and the cytological satellites), while in the 15ph+s+ (fig. 1*b*), the nature of the fluorescent satellites is still not known, although they do not hybridize with any of the four major satellite cRNAs [10].

Evidence about the distribution of satellite DNA is also demonstrated by case S in which a deletion of the short arm makes this chromosome apparently telocentric. No satellite or rDNA can be detected in this chromosome, although its familial distribution means that it obviously has a functioning centromere; this confirms that all the satellite DNA is normally in the short arm.

What, if anything, does all this evidence about the quantitative variability of satellite DNAs, coupled with the close association with rDNA, say about the vexed question of satellite DNA function? First, and most important, the only chromosomal units (counting a Robertsonian translocation as a single unit) in which no satellite DNA can be detected are the 13p− in cases O and S; here it is possible that satellite DNA still exists, below the level of detection. Bearing this in mind, suggested functions such as chromosome pairing or regulation of condensation are still possible but unlikely. We previously suggested that the function may be associated with nucleolus organization and rRNA transcription [16]. The 13p− of O and S certainly lack rDNA as do P and Q and the 15p− of R, and O and S may also lack satellite DNA. None of these chromosomes appear to be involved in chromosomal associations, and a priori one might expect this from the absence of rDNA. However, in *Gorilla*, the large acrocentric chromosomes that lack rDNA but contain satellite DNA [16] do participate in chromosomal associations [17], and since the small NOR-carrying chromosomes are involved in the same associations (which are presumed to be nucleolar relics), it may be the absence of satellite DNA rather than that of rDNA that excludes the 13p− from such associations. Mitchell et al. [18] have shown that single buoyant density satellites, such as human satellite III, can be extremely heterogeneous in that they can include a mixture of repeated sequences that are not obviously related to each other. Discussing functions like these in terms of total satellite DNA or, even worse, in terms of total heterochromatic chromosome regions [19] may be misleading. If satellite DNAs have a function(s) in man (and this is in dispute), the fact that the satellite DNA sequences are conserved between man and the great apes [18, 20, 21] might suggest that the function is sequence related. The existence of chromosome-specific subfractions of satellite DNAs [19, 22–24] supports such a hypothesis.

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