Atypical Acrocentric Chromosomes in Negro and Caucasian Mongols

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THIS STUDY WAS initially undertaken in order to determine the frequency of translocation among Negro mongols as compared to Caucasian mongols. During the early phases of the investigation, however, chromosomes showing morphologic variation, particularly in the D and G groups, were noted in the karyotypes of several mongols. Therefore, we extended the study and investigated the frequency of atypical acrocentrics in a series of normal individuals.

METHODS

Subjects. Twenty Negro mongols were studied, comprising all Negro mongols in three large Michigan state institutions who had not previously been karyotyped in our laboratory. Four were female and 16 were male, with ages ranging from eight to 36 years, and a median age of 13. In addition, 20 institutionalized Caucasian mongols matched for age and sex were included in the study. None of these 40 subjects had mongol sibs.

Twenty Negro and 20 Caucasian males ascertained solely on the basis of their racial identity and sex served as normal control subjects. The family history of these individuals is not known to us.

No attempt has yet been made to contact relatives of the experimental subjects in order to study the intrafamilial transmission of aberrant chromosomes.

Procedure. Short term leukocyte cultures were prepared using a modification of the method of Moorhead *et al.* (1960). For the mongol subjects, karyotyping was performed by direct examination of coded slides under phase contrast optics. At least 20 counts were done on each individual; in the majority of cases, over 20 karyotypes were prepared, and photomicrographs were then taken of representative cells. For the control series, photomicrographs of at least ten cells of each subject were available from a previous study done in this laboratory (Cohen *et al.*, 1966).

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Scoring. Because of the cell-to-cell and culture-to-culture variation in the size and appearance of the short arms of acrocentric chromosomes, each cell was scored independently. Thus, the acrocentrics within a given cell served as controls for each other. Before an individual was judged to possess an atypical acrocentric, at least 75% of the cells examined had to show consistency as to the presence of such a chromosome. In most cases, the atypical chromosome was identifiable in every cell.

The foregoing karyotype studies of both normal controls and mongol patients were performed by one of us (M.N.S.). For each individual suspected of possessing an atypical acrocentric chromosome, coded photographs of several representative cells were then scored independently by four experienced cytologists in our laboratory. A consensus of at least three of four observers determined the final decision on each subject.

RESULTS

Frequency of Translocation and Mosaicism

There were no translocations detected in the 40 mongols studied. One Caucasian mongol, a 12-year-old male, was found to be a mosaic (Table 1, SN).

Frequency of Atypical Acrocentric Chromosomes

Two Caucasian controls each had a deficient amount of chromatin on one D group chromosome. In one of these individuals, the chromosome was apparently telocentric (Fig. 1, CO); in the other, the heterochromatic stalks and satellites were missing (Fig. 1, BW).

The most significant finding in this study was the occurrence of 11 individuals with prominent short arms and/or satellites on the acrocentric chromosomes. Eight of 40 mongols (six Negroes and two Caucasians) had such a chromosome. In contrast, three of 20 Negro controls and none of 20 Caucasian controls had enlarged short arms or satellites. Six cases involved a D group chromosome (Fig. 1) while five cases involved G group chromosomes (Figs. 2, 3).

The chromosomes with enlarged short arm regions were capable of entering into associations with the normal acrocentrics, whereas the acrocentrics with deficient short arm material were never seen in associations.

Pertinent information on these individuals is given in Table 1.

Morphology of the Atypical Acrocentric Chromosomes

Although the presence of an atypical chromosome was obvious in almost all technically good cells, the precise morphology of the short arm material appeared to vary from cell to cell. For example, in one cell "double" tandem satellites might be observed while in another cell of the same individual the short arm would appear as a prominent knob. If the structural nature of the short arm enlargement is specific and identical in each cell of the same individual, technical factors as well as the state of contraction could account for the variations in morphology. Because of this variability, however,

TABLE 1. INDIVIDUALS POSSESSING AN ATYPICAL KARYOTYPE

Classification	Sex	Age	Maternal age	Clinical findings	Family history	Chromosomal abnormality*	Remarks
Atypical acrocentric chromosome							
Negro mongols							
JJ	Male	17	18	Cretin	One retarded sibling	D+	
TR	Male	9	20			D+	Prominent secondary constriction in Chromosome 9
AW	Female	29	42			D+	Inconstant karyotypic abnormalities (see text)
RC	Male	36	39			D+	
GB	Male	9	33	Congenital cyanotic heart disease	One retarded sibling	G+	
BC	Female	13	37			2G+	Marked acrocentric "bridges"
Caucasian mongols							
DS	Male	9	31		Two paternal uncles in institutions	G+	
TB	Male	26	44			G+	
Negro controls							
AD	Male					G+	
BR	Male					D+	
WI	Male					D+	
Caucasian controls							
BW	Male					D−	
CO	Male					D−	
Mosaicism							
Caucasian mongol							26/50 = 47 tri-21 XY
SN	Male	12	22				24/50 = 46 XY
Asymmetric pair 16							
Negro mongol							
WL	Male	12	43				

*+ = apparent extra chromatin material on short arm of acrocentric.
− = apparent loss of chromatin material from short arm of acrocentric.

it could not be concluded whether the enlarged area involved the satellite region only or other parts of the short arm.

One case (BC) deserves special comment. In most cells of this female Negro mongol, two group G chromosomes were found to have an abnormal morphology (Fig. 3, first row). In some cells, these two chromosomes looked metacentric, with the general appearance of small group F chromosomes; in other cells, a stretched secondary constriction region was visible in the short arms and the satellites appeared to be unusually large. Rarely, either one G chromosome only or three G chromosomes appeared abnormal (Fig. 3, second row). In one cell a cross-like configuration between the short arms of two G group chromosomes was noted, suggesting a chromatid exchange

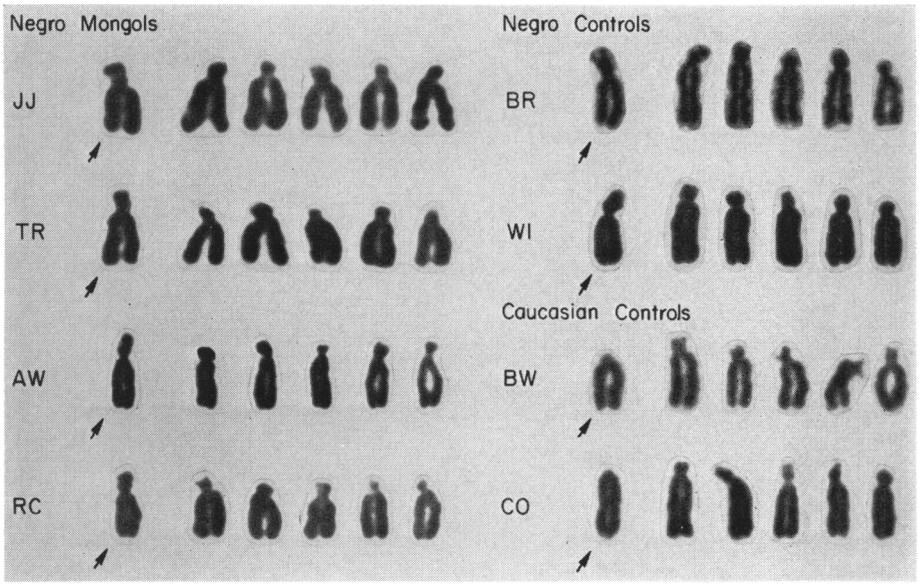

FIG. 1. This composite illustrates the D group chromosomes in a representative cell from each of eight individuals found to have an atypical D chromosome. The arrow points to the atypical D chromosome in each cell.

is taking place (Fig. 3, third row). This phenomenon happens rarely in normal cells. If a similar event occurred in this individual in an earlier cell division, this could account for the observation of three cell lines with one, two, or three small acrocentrics with prominent short arms.

Other Chromosome Aberrations Found in the Mongol Subjects

One Negro mongol possessed an enlarged chromosome 16 (Fig. 4). In some cells, the secondary constriction was particularly prominent and the long arms appeared lengthened as well; in other cells, the constriction was not evident and only the long arms appeared elongated.

One Negro mongol with 47 chromosomes, including an atypical D chromosome (Fig. 1, AW), had in addition a number of unusual cells. Several cells had 48 chromosomes including an extra group C chromosome, while other cells had 47 chromosomes plus a long acentric fragment. In one cell of 40, a D group chromosome possessed long arms twice as long as normal, producing a striking marker chromosome. This patient has no clinical or laboratory evidence of a hematologic abnormality at the present time.

The results are summarized in Table 1.

DISCUSSION

Frequency of Translocation Among Negro Mongols

The frequency of translocation among patients with mongolism has been estimated to be about 3.5% (Polani *et al.*, 1965). The data on which this figure is based do not include any differentiation among racial groups. Most

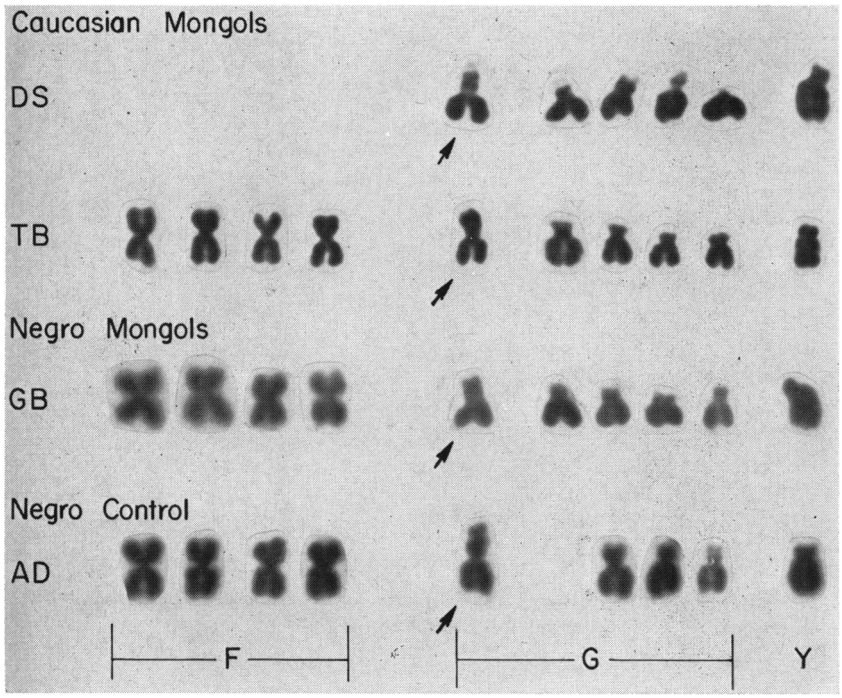

FIG. 2. This composite illustrates the G group chromosomes in a representative cell from four of the five individuals found to have an atypical G chromosome. The arrow points to the atypical chromosome.

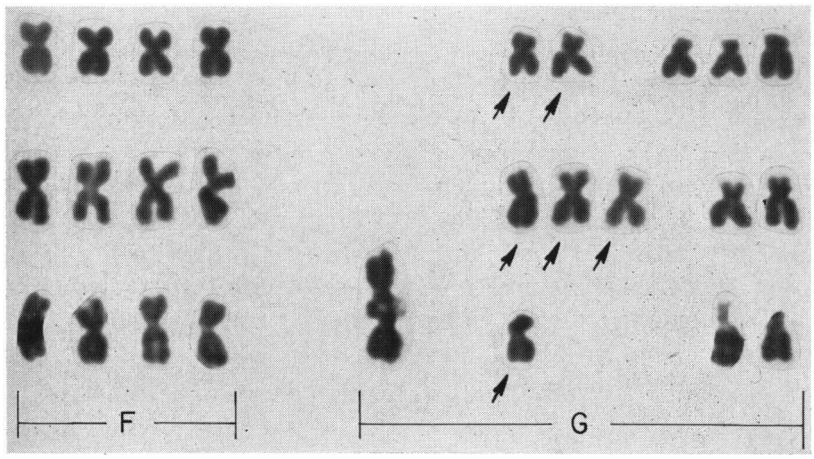

FIG. 3. These are the F and G group chromosomes from three different cells of the fifth individual with atypical G chromosomes (BC). In most cells, two group G chromosomes were atypical (Row 1). Rarely, three group G chromosomes were atypical (Row 2). A cross-like configuration between the short arms of two group G chromosomes was noted in one cell (Row 3). The arrows point to the atypical chromosomes.

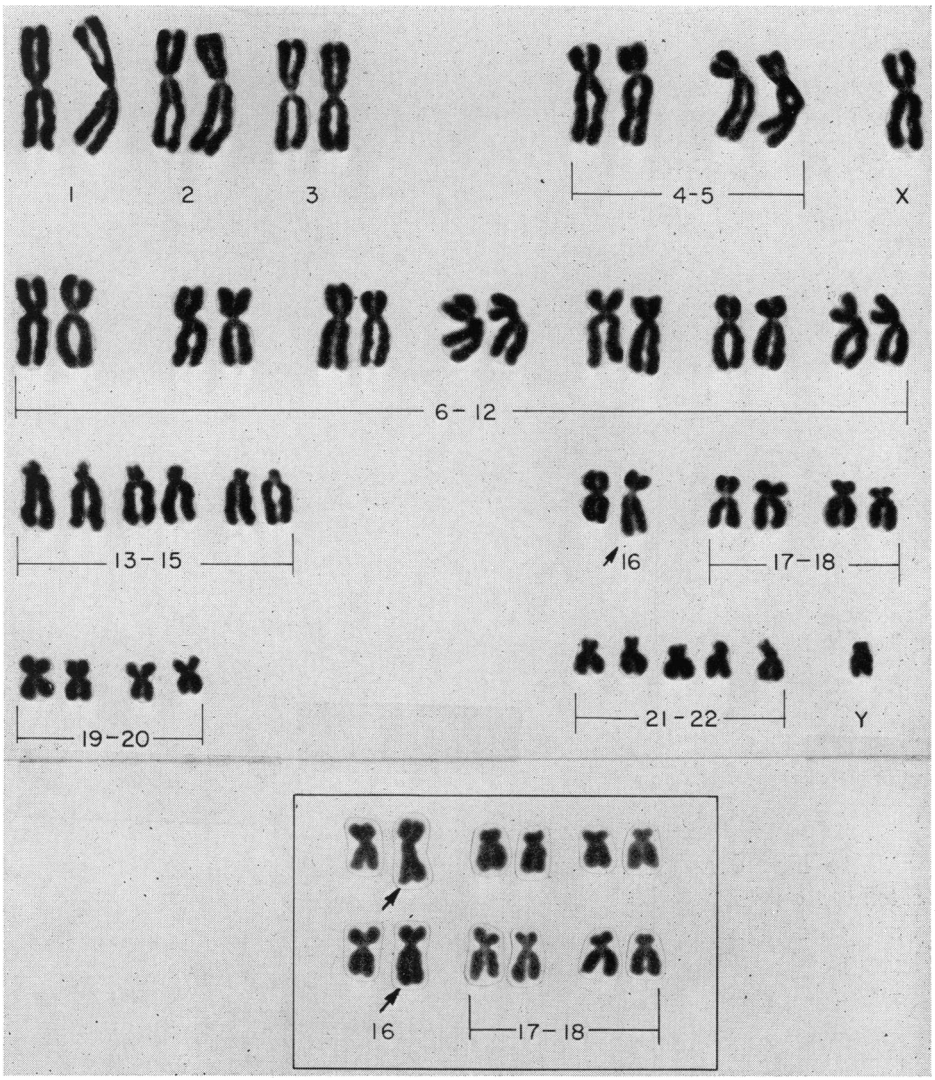

FIG. 4. This is a karyotype of the Negro mongol possessing an atypical chromosome 16. The inset demonstrates the variable morphology of the atypical chromosome. The arrow points to the atypical 16 of each cell.

studies on this subject have been done on predominantly Caucasian populations. Thirty-one mongols whose race is specified as Negro, however, are known to us from the literature (Table 2). Of these, 25 are trisomic and six are translocation mongols. We could not confirm this high frequency of translocation among Negro mongols. The reports in the literature may reflect a spuriously high frequency because of sampling error and ascertainment bias.

TABLE 2. NEGRO MONGOLS FOUND IN LITERATURE SURVEY

Reference	Number of Negro mongols studied	Number of mongols with trisomy 21	Number of mongols with translocation	Other findings
Hayashi (1962)	3	2	1 D/G	
Migeon *et al.* (1962)	1		1 G/G	Centric fragment also present
Sherz (1962)	1		1 D/G	
Dekaban *et al.* (1963)	6	6		One had an "oversized G;" one had a long Y and an "abnormal 18"
Mercer *et al.* (1963)	1	1		Mosaic: 47/48 extra G/extra G and C
Walker *et al.* (1963)	2	1	1 D/G	Tri-21 mongol is uncle of translocation mongol
Tompkins (1964)	2	2		
Wilton (1964)	6	6		One mosaic: 46 normal/47 tri-21
Migeon (1965)	1	1		This individual is also XYY
Weinstein *et al.* (1965)	8	6	1 D/G 1 G/G	

Frequency of Atypical Acrocentric Chromosomes

There have been numerous reports of atypical short arms or enlarged satellites on the group D or group G chromosomes in patients with a variety of clinical diagnoses. Many of these acrocentric chromosomes are known to have been inherited from physically and mentally normal parents (e.g., Cooper and Hirschhorn, 1962). Several investigators have attempted to estimate the frequency of atypical acrocentric chromosomes in either a normal population or a selected series of abnormal individuals. The results of several such surveys, including ours, are summarized in Table 3.

Our study revealed a higher frequency of acrocentric abnormalities among mongol subjects than has been previously reported in several large mongol surveys (Hayashi, 1962; Sergovich, 1964; Hamerton *et al.*, 1965). Dekaban *et al.* (1963), however, observed an "oversized" small acrocentric in three of 14 mongols tested. More recently, Edgren *et al.* (1966) reported that five of seventy-three patients with mongolism had large satellites on a group D or group G chromosome. Surveys of "normal populations" (Chandra and Hungerford, 1963; Court Brown *et al.*, 1965) have reported a frequency of about 2% atypical acrocentrics, which, again, is lower than the findings in our series of normal control subjects.

In any study of this type, the difficulty lies in setting the boundary between normal and abnormal variations in size and morphology. Using present

techniques and methods of analysis, such decisions are necessarily arbitrary. We have tried to compensate for this by obtaining the independent judgments of four observers well familiarized with chromosome analysis. Other workers in other laboratories may well have differing criteria on which they base their decisions. Since the methods of preparation and criteria for decision were identical for all the karyotypes we examined, however, the comparative results among the different groups in our study should be valid.

Because our sample sizes are too small for statistical analysis, no definitive conclusions can be drawn from the data. Our findings, however, can be interpreted in several ways:

1. The frequency differences which we observed may be spurious because of small sample size. In other words, acrocentric variability could occur to an equal extent in both normal and abnormal individuals of both racial groups and be unrelated to aneuploidy or race.

2. Since nine of 40 Negroes, as compared with two of 40 Caucasians, had acrocentrics with prominent short arm regions, the possibility must also be entertained that we have observed a racial polymorphism. In certain mammals, autosomal polymorphism is not uncommon (Matthey, 1965; Yosida and Amano, 1965; Cohen and Pinsky, 1966). In the human species, there is evidence for racial and ethnic differences in the length of the Y chromosome (Cohen *et al.*, 1966).

3. Since eight of 40 mongols and three of 40 controls had prominent short arms on an acrocentric chromosome, another hypothesis to explain our observations is that small structural variations do, indeed, bear a relation to nondisjunction.

These latter two interpretations need not be mutually exclusive. Populations with differing frequencies of small structural variants may have different predispositions to aneuploidy.

Structure of Atypical Acrocentric Chromosomes

Cooper and Hirschhorn (1962), as well as others, have proposed alternative hypotheses to explain the morphologic variation seen in the acrocentric chromosomes. They suggested that an enlarged short arm region may represent either a true structural rearrangement, such as translocation or duplication, or may be due to genetically determined phenomena in the heterochromatin.

We favor the concept that a true structural change has occurred in these chromosomes. There is theoretical support for such a view. Several authors (Ferguson-Smith and Handmaker, 1963; Ohno *et al.*, 1961) have suggested that part of the short arm region of the acrocentrics is composed of heterochromatin that is involved in nucleolar organization. Hamerton (1962) has cited studies demonstrating that the presence of a nucleolar organizer in a chromosome predisposes that region to breakage and structural rearrangement. We did not observe unusual prominence of the secondary constriction regions of chromosomes 1, 9, or 16 in the subjects with an atypical acrocentric chromosome. Thus, we do not feel that our findings are due to despiralization or abnormal coiling producing a generalized prominence of heterochromatin.

TABLE 3. FREQUENCY OF ATYPICAL ACROCENTRICS WITH PROMINENT SHORT ARMS AND/OR ENLARGED SATELLITES FOUND IN RECENT SURVEYS

	Authors	Subjects	Population studied	Chromosome affected	Number of individuals or families with the atypical acrocentric	Frequency of atypical acrocentrics	
Normal individuals not ascertained through patients							
	Chandra and Hungerford (1963)		American	D	1	1/50	2%
	Court Brown *et al.* (1965)		Scottish	D	2	7/438	2%
				G	5		
	Starkman and Shaw (this study)		American Negro (N) and Caucasian (C)	D	2 (N)	3/40	8%
				G	1 (N)		
Selected individuals							
	Miller *et al.* (1962)	(a) Individuals with sexual or other congenital abnormalities (b) Relatives of such individuals	American	Not stated	Not stated	10/40	25%
	Hamerton *et al.* (1965)	(a) Mongols (b) Phenotypic "mongols" with normal karyotypes (c) Families of the index patients	English	D	2	5/208	2%
				G	3		
	Edgren *et al.* (1966)	Mongols	Finnish	D	2	5/73	6.8%
				G	3		
	Starkman and Shaw (this study)	Mongols	American Negro (N) and Caucasian (C)	D	4 (4N)	8/40	20%
				G	4 (2N + 2C)		

TABLE 4. MATERNAL AGE AT BIRTH OF THE MONGOL CHILD

<30 years	>30 years
17	*31
*18	32
20	33
*20	*33
20	34
21	34
21	37
21	*37
21	37
22	37
22	*39
22	39
22	*42
23	42
24	43
24	†43
24	*44
25	45
26	46
27	
28	

*Mother of mongol with atypical acrocentric.
†Mother of mongol with atypical 16.

Differential coiling and true structural change, however, need not be mutually exclusive. An alteration in coiling restricted to one heterochromatic region might well be a reflection of a structural abnormality at that site.

Maternal Age Distribution

In an effort to determine whether possession of an atypical acrocentric could be associated with the well-known maternal age effect in mongolism, we have listed the maternal ages at the birth of the 40 mongols studied (Table 4). Although 16 of the 40 mongols were born to women 21 to 30 years of age, not a single instance of an atypical acrocentric was found. In contrast, six of 19 mongols born to women over 30 years of age and two of five mongols born to women 20 years of age or younger were found to have such an aberrant chromosome. If the mongol owed his aneuploidy to an *inherited* atypical acrocentric, then no such maternal age association would have been expected.

Ferguson-Smith (1964), in a study of pachytene chromosomes of human primary spermatocytes, demonstrated that the nucleoli were associated with the terminal chromomeres of the short arms of the acrocentrics. He observed numerous examples of multiple associations between these nucleolar bivalents. Ohno *et al.* (1962) have shown that human primary oöcytes remain arrested in the dictyotene stage of first meiotic prophase until ovulation. It thus seems reasonable to speculate that, after a 30 to 40 year period of association, the

acrocentric chromosomes may have an increased opportunity for structural rearrangements. These structural changes, either arising *de novo* during gametogenesis or present as a variant chromosome in all cells, may then predispose the chromosomes to nondisjunction.

SUMMARY

Because of several reports suggesting that translocation mongolism is more frequent among Negroes than among Caucasians, the karyotypes of 20 Negro and 20 Caucasian mongols were studied. Although there were no translocations found, morphologic abnormalities in the short arm or satellite region of the acrocentric chromosomes occurred in six Negro and two Caucasian mongols. In a control series of 20 Negro and 20 Caucasian males, three Negroes showed similar abnormalities, while two Caucasians had an apparent deletion in the short arm of one acrocentric. One Negro mongol had marked asymmetry of chromosome pair 16. The maternal ages of the nine mongols with cytologic variations were either 20 years and below or 31 and above. The significance of structural variation in the short arm regions of the human acrocentric chromosomes is discussed.

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