A Search for Autosomal Linkage in a Trisomic Population: Blood Group Frequencies in Mongols

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SEVERAL RECENT ADVANCES in human cytogenetics have provided different avenues of approach to the mapping of autosomal genes. The discovery of inherited morphological variants of the chromosomes furnishes cytologic markers which may be used in tests for linkage. For example, Ellis and Penrose (1961) described a kindred in which a giant satellite is segregating and supplied serological data on the individuals who were karyotyped. Pedigrees with a chromosomal aberration are also beginning to yield autosomal linkage data. Penrose and Delhanty (1961), Shaw (1962), and Atkins, O'Sullivan and Pryles (1962) have performed blood typing in families in which a translocation chromosome is segregating. The discovery of trisomy in mongolism by Lejeune, Gautier and Turpin (1959) has paved the way for another approach to human autosomal mapping. This method, which would utilize statistical procedures rather than direct cytologic observations and classical genetic linkage tests, involves a search for a deviation in the frequency distribution of certain phenotypes in the trisomic population compared to the general population (Bateman, 1960).

This paper describes an attempt to determine whether any of eight markers could be placed on chromosome 21 by typing the blood and saliva of a large sample of mongols. Several authors have reported blood groups of mongols for other reasons, and their findings will be summarized.

THEORETICAL CONSIDERATIONS

Let us assume there are two alleles, A and A', which are present with equal frequency and exhibit no dominance. Under conditions of equilibrium, 50 per cent of the individuals will be heterozygous (AA'). Now, if a trisomic population draws *randomly* from this 50:50 gene pool, which would occur if the gene is close to the centromere and the chromosome undergoes nondisjunction in the *first* meiotic division, then the trisomic phenotype frequencies could be predicted (assuming no dosage effect): The heterozygote class would increase in frequency to 75 per cent ($3p^2qAAA' + 3pq^2AA'A'$).

It should be pointed out here that the basic phenomenon which would cause a shift in the phenotype frequencies in the trisomic population is that *both* alleles of the parent undergoing nondisjunction must be found in the trisomic

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TABLE 1. THE PHENOTYPE RATIOS EXPECTED IN TRISOMY FOR A TWO-ALLELE SYSTEM WITH NO DOMINANCE, WHERE $p_A = q_{A'}$

Mating types		Relative frequency	Expected diploid ratios			Expected trisomic ratios (No crossing-over)							
						First meiotic nondisjunction				Second meiotic nondisjunction			
Normal parent x	Nondisjunctional parent		AA	AA'	A'A'	AAA	AAA'	AA'A'	A'A'A'	AAA	AAA'	AA'A'	A'A'A'
AA	x AA	1	1			1				1			
AA	x AA'	2	1	1			2			1	1		
AA	x A'A'	1		1				1				1	
AA'	x AA	2	1	1		1	1			1	1		
AA'	x AA'	4	1	2	1		2	2		1	1	1	1
AA'	x A'A'	2		1	1			1	1			1	1
A'A'	x AA	1		1			1				1		
A'A'	x AA'	2		1	1			2				1	1
A'A'	x A'A'	1			1				1				1
Genotype ratios			4	8	4	2	6	6	2	4	4	4	4
Phenotype ratios			1A	2AA'	1A'	1A	6AA'		1A'	1A	2AA'		1A'
Results						Increase in proportion of heterozygotes				No shift in phenotype frequencies			

offspring. If only *one* parental allele is represented in duplicate, then that child's genotype would not contribute to a shift in phenotype frequencies. Both alleles are contributed when first meiotic nondisjunction occurs and there is no crossing-over between the locus and the centromere of the chromosome involved. Second meiotic nondisjunction (with no crossing-over) and post-fertilization mitotic nondisjunction resulting in an undetected mosaic will always give rise to a duplication of one parental allele in the trisomic with no change in phenotype frequencies resulting. First meiotic nondisjunction with crossing-over between the locus and the centromere would result in the recovery of some trisomics who possessed both alleles of the parent who contributed the extra chromosome and some trisomics with one parental allele represented in duplicate. It is not known to what extent these chromosomal mechanisms are responsible for the appearance of human trisomics, but such occurrences would depress the magnitude of the shift, and the observed phenotype frequencies in the trisomic population would lie somewhere between the diploid frequencies and the theoretical maximum trisomic frequencies. Table 1 gives an example of what would happen to the trisomic phenotype frequencies in the two limiting cases of first meiotic nondisjunction and second meiotic nondisjunction with no crossing-over.

While the chromosome mechanisms determine whether or not there will be a shift, the gene frequencies of the parental population must also be considered because they contribute to the magnitude of the shift. Thus the MN antigens are an example of a two-allele system with no dominance where the diploid heterozygote class is approximately 50 per cent and an increased frequency of the MN phenotype in the trisomic population ($3p^2qMMN + 3pq^2MNN$) would be relatively easy to detect. On the other hand, when the frequencies of the two alleles are quite unequal, the demonstration of a deviation of the phenotype frequencies in the trisomics would require an extremely large sample. For example, the *K* allele of the Kell blood group locus has a gene frequency of

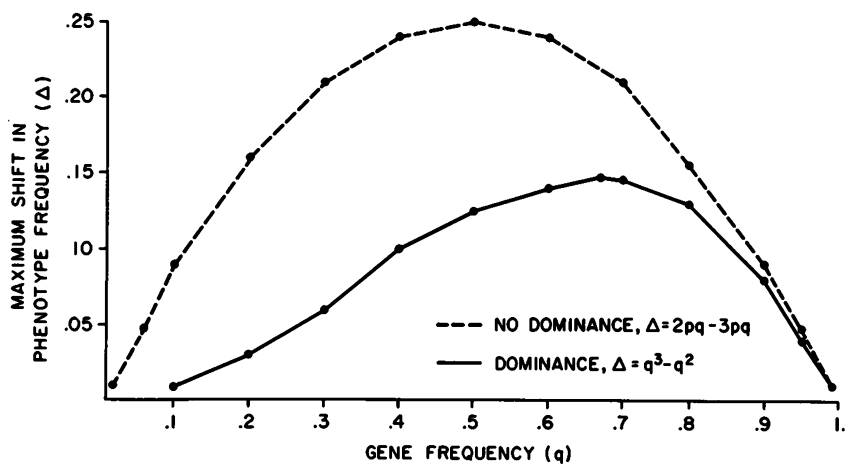

FIG. 1. Maximum shifts expected in phenotype frequencies for varying gene frequencies.

about 5 per cent. Kell-positive individuals ($p^2KK + 2pqKk$) comprise about ten per cent of the general population. Trisomy (p^3 *KKK* + $3p^2q$ *KKk* + $3pq^2$ *Kkk*) would lead to a maximum of a five per cent increase in Kell-positives.

Fig. 1 illustrates the magnitude of the maximum shift expected in phenotype frequencies with varying gene frequencies. For a system with no dominance the greatest shift would be observed if the two alleles have equal frequencies. In the case of dominance, a maximum detectable shift occurs if the recessive allele has a frequency of 0.67. In either case, whether dominance exists or not, these frequencies apply only if: (1) trisomy arises by first meiotic nondisjunction, (2) the locus is closely linked to the centromere, and (3) dosage effects do not interfere with the phenotypic classification.

The discussion up to this point has dealt with specific instances of gene frequency changes. A more general treatment of the problem is possible, however, and has been kindly suggested by Dr. James F. Crow. Dr. Crow was also gracious enough to permit its inclusion in this paper, as follows:

Let x represent the proportion of homozygous gametes among those gametes with an extra chromosome from a diploid heterozygous parent; then $1 - x$ will be the proportion of heterozygous disomic gametes from the same parent. The array of matings and the expected proportions of phenotype classes among the trisomic zygotes produced from a randomly mating diploid population with gene frequencies p and q may then be derived. These are given in table 2. Now, if the expected trisomic classes are compared to the parental diploid classes, the ratios given in table 3 will result.

It may be seen in table 3 that whatever the gene frequencies of the two alleles in the parental population the maximum *increase* in the heterozygote class among the trisomics (where $x = 0$) is 50 per cent of the diploid value.

TABLE 2. HAPLOID AND DISOMIC GAMETES AND RESULTANT TRISOMIC ZYGOTES ARISING IN A RANDOMLY MATING DIPLOID POPULATION, FOR A TWO-ALLELE SYSTEM WHERE P = FREQUENCY OF GENE A AND Q = FREQUENCY OF GENE A′. SEE TEXT FOR DEFINITION OF X

Gametes with extra chromosome	Expected proportion	Haploid gametes	Proportion	Haploid gametes	Proportion
		A	p	A′	q
AA	$p^2 + pqx$	AAA	$p^2(p + qx)$	AAA′	$pq(p + qx)$
AA′	$2pq(1 - x)$	AAA′	$2p^2q(1 - x)$	AA′A′	$2pq^2(1 - x)$
A′A′	$q^2 + pqx$	AA′A′	$pq(q + px)$	A′A′A′	$q^2(q + px)$

TABLE 3. SUMMARY OF ZYGOTE CLASSES GIVEN IN TABLE 2 AND COMPARISON OF DIPLOID PARENTAL FREQUENCIES TO EXPECTED TRISOMIC ZYGOTE FREQUENCIES

Trisomic population genotype	Proportion	Diploid population genotype	Proportion	Ratio trisomic/diploid
AAA	$p^2(p + qx)$	AA	p^2	$p + qx$
AAA′	$3p^2q + pqx(q - 2p)$			
+ AA′A′	$3pq^2 + pqx(p - 2q)$			
AAA′ + AA′A′	$pq(3 - x)$	AA′	$2pq$	$\frac{3}{2} - \frac{1}{2}x$
A′A′A′	$q^2(q + px)$	A′A′	q^2	$q + px$

Furthermore, the maximum *decrease* in the homozygote classes among trisomics is a fraction, p or q, of the diploid value.

The quantity x has a cytological interpretation. If homozygous gametes of a heterozygous parent result from first division nondisjunction with a crossover between the gene and the centromere, then $x = c$, where c is the frequency of such crossing-over in nondisjunctional meiosis. For short distances, the parameter 100X becomes the genetic map distance of the gene from the centromere. However, if the homozygous exceptional gametes are due to second division nondisjunction, then $X = 1 - 2c$. If $X = 1$, then $c = 0$ and the expected phenotype frequencies are those observed in the diploid parental population.

The above considerations concern loci with two alleles. Analyses of trisomy at loci with multiple alleles (e.g., the Rh locus) are much more complex and would require discussion in greater detail. The ABO locus may be regarded as a three-allele system (A, B, O) or four-allele system (A_1, A_2, B, O) depending on the serological typing.

In summary, the detection of a shift from the diploid phenotype frequencies in a trisomic population will depend upon: (1) the number of alleles at a given locus, (2) dominance relations, (3) dosage effects, (4) gene frequencies in the parental population, (5) map distance between the locus and the centromere and (6) stage at which the disjunctional error occurs.

MATERIALS AND METHODS

The Mongol Population. The 793 mongols reported in this study were inpatients at four state institutions for the mentally retarded:

1. Plymouth State Home and Training School, Plymouth, Michigan.
2. Coldwater State Home and Training School, Coldwater, Michigan.
3. Lapeer State Home and Training School, Lapeer, Michigan.
4. Orient State Institute, Orient, Ohio.

The map in Fig. 2 gives the geographical location of these institutions and the town chosen for the control frequencies. For two of the institutions, Plymouth and Coldwater, finger-prick and saliva specimens were tested in the laboratory. At the other two, Lapeer and Orient, slide typings for the ABO system only were performed at the institution.

Patients were excluded from the study if the diagnosis was uncertain or if they were not Caucasian. The latter exclusion was made because the control group was entirely Caucasian. There was no attempt to exclude patients born at a relatively young maternal age, although some justification could be made for such a breakdown, in an attempt to exclude those mongols resulting from causes other than ordinary trisomy due to nondisjunction in the aging oocytes.

The Control Population. The choice of a control population for blood group frequency studies has been the subject of much debate. Groups which have been used include blood bank donors, hospitalized patients, age-matched groups, name-matched groups, and sibs. It is obvious that the heterogeneous nature of the present mongol population, being drawn from Michigan and Ohio, precludes the possibility that a single, randomly collected control group could effectively be used without raising objections.

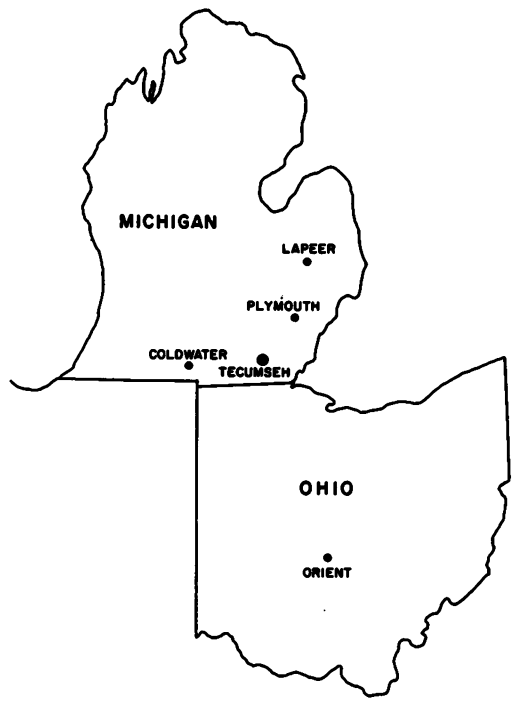

FIG. 2. Geographical location of the four institutions and the control community.

The control sample in this study was taken from a Southern Michigan town (Tecumseh) with a population size of about 10,000. This community is the focus of a large-scale health survey by the School of Public Health of the University of Michigan under the direction of Drs. Thomas Francis and Frederick H. Epstein. Blood and saliva group data were available on 754 married couples in whom the wives had reached age 40, obtained in conjunction with a fertility study to be reported by Reed, Gershowitz and Soni. It was felt that this sample would be comparable to that of the parents of the mongols under study and thus would essentially represent the gene pool from which the mongols were drawn.

It is, of course, possible that this control population is not representative. One objection, mentioned above, is the limited geographical distribution of the controls compared to that of the mongols. To test the uniqueness of the control group, the ABO frequencies were compared with two other published control series, one in Michigan and the other in Iowa, which is also a Midwestern agricultural state. These comparisons are shown in table 4. It is evident that there is close agreement among the three groups.

Another objection concerning the controls is that the parents of mongols may not be a representative population group. This does not refer to the well established maternal age effect, but to the growing body of evidence that nondisjunction is not a random event and may take place more frequently in certain predisposed individuals. Support for this idea comes from the reports of related

TABLE 4. COMPARISON OF ABO FREQUENCIES IN CONTROL POPULATION USED IN PRESENT STUDY WITH TWO OTHER CONTROL SERIES

Controls	Number in series	Percentage frequencies A	B	AB	O	Reference
Tecumseh, Mich. married couples	1508	41.2	9.5	3.7	45.6	Present study.
Jackson, Mich. married couples	1116	41.7	8.4	3.6	46.3	Reed & Ahronheim, 1959.
Iowa, Caucasian blood donors	6313	41.6	9.0	3.7	45.8	Buckwalter *et al.*, 1957.

individuals with different chromosome abnormalities (e.g., Miller, Breg, Schmickel and Tretter, 1961) as well as the discovery of individuals with trisomy for both an autosome and a sex chromosome (e.g., Ford *et al.*, 1959; Harnden, Miller and Penrose, 1960) and individuals who are tetrasomic or pentasomic for the sex chromosome pair (e.g., Barr and Carr, 1960; Fraser, Boyd, Lennox and Dennison, 1961). All of these cases require the assumption of more than one nondisjunctional event. Although there is no reason to postulate that these exceptional individuals (in whom nondisjunction has occurred) would differ in their blood groups from those of the general population, acceptance of the objection as valid would make it mandatory that the control group be the parents of mongols, and sufficient data from these individuals are not yet available.

Serological Methods. Blood specimens collected from Plymouth and Coldwater were tested by the tube method using washed, 2 per cent saline suspensions of red blood cells. The antisera used were those which detected the following antigens: A, A_1, B, M, N, Mg, S, s, C, C^w, c, D, E, e, P, K, k, Kp^a, Kp^b, Fy^a, Jk^b, V^w, and Wr^a. All D-negative bloods were tested for D^u and all P_1-negative bloods were tested with anti-P + P_1. Only E-positive bloods were tested for e, S-positive bloods tested for s, and M or N homozygotes tested for Mg. Tests for Mg, C^w, Kp^a, Kp^b, V^w, and Wr^a were performed only as part of a routine search for bloods which differ from those of the general population. Antigens present in (or absent from) the great majority of bloods are obviously useless in a population search for linkage.

Saliva specimens were collected in the following manner: Each patient was given a cotton swab on an applicator stick and told to chew it for several minutes. In the case of infants or patients unable to comprehend the instructions the mouth was wiped with the swab. For the most part, obviously wet swabs were collected; a few were noted to be quite dry. Whenever possible, the swab was squeezed with a small press and a few drops of saliva collected in a small test tube. If a specimen could not be obtained in this manner, 0.5 ml. saline was flushed through the swab. All the tubes were placed in a boiling water bath for ten minutes. Raw saliva was tested for ABH secretion at a 1/20 dilution whereas the "salivas" obtained only after the addition of saline to the swab were tested without further dilution.

In a number of cases, secretor typing of the salivas presented some difficulties. Non-specific inhibition was noted in 21 instances: salivas of 19 type O and 2 type B persons inhibited anti-A. All 21 were "salivas" in which a small amount of saline had been added to the cotton swab and the fluid obtained had

TABLE 5. RESULTS OF SEROLOGICAL TESTS OF 370 MONGOLS[1] AND 1508 CONTROLS[2]. PERCENTAGE FREQUENCIES IN PARENTHESES

Phenotype	Mongols		Controls		Comparison	χ^2	P
A_1	131 (35.4)		474 (31.4)				
A_2	39 (10.5)		147 (9.8)				
B	39 (10.5)		144 (9.6)				
A_1B	7 (1.8)		39 (2.6)				
A_2B	5 (1.4)		16 (1.1)				
O	149 (40.3)		688 (45.6)		O vs. non-O	3.446	.10 — .05
M	102 (27.6)		445 (29.5)				
MS		19	*				
MSs		58					
Ms		25					
MN	186 (50.3)		750 (49.7)				
MNS		13					
MNSs		81					
MNs		92					
N	82 (22.2)		313 (20.8)				
NS		1					
NSs		19					
Ns		62			MN vs. M + N	0.034	.90 — .80
R_1r, R_1^wr		130,1		520,12			
R_1R_1, $R_1^wR_1$		62,4		231,18			
R_1R_2, $R_1^wR_2$		50,2		205, 7			
R_2r		50		193			
R_2R_2		9		27			
R_0		4		23			
RzR_1, RzR_2		1,1		2, 0			
Total rr	56 (15.1)		268 (17.8)				
rr, R'r, R"r		44, 2, 1		228, 14, 8			
R_1^ur, $R_1^uR_1$, R_2^ur, R_0^u†		2, 0, 2, 5		8, 2, 2, 6	D+ vs. D—	1.471	.30 — .20

TABLE 5. Cont.

P+	273 (73.8)	1135 (75.3)			
P—	97 (26.2)	373 (24.7)	P+ vs. P—	0.348	.70 — .50
Fy^a+	259 (70.0)	999 (66.3)			
Fy^a—	111 (30.0)	508 (33.7)	Fy^a+ vs. Fy^a—	1.849	.20 — .10
KK	1 (0.3)	4 (0.3)			
Kk	25 (6.8)	121 (8.0)			
kk	344 (93.0)	1383 (91.7)	KK + Kk vs. kk	0.640	.50 — .30
Jk^b+	187 (66.1)	112 (65.1)**			
Jk^b—	96 (33.9)	60 (34.9)	Jk^b+ vs. Jk^b—	0.023	.90 — .80
ABH secretor	282 (76.8)	1152 (76.7)			
Non-secretor	85 (23.2)	349 (23.3)	Sec.+ vs. Sec.—	0.001	.98 — .95

[1]Mongol's not tested: Jk^b — 87; Sec. — 3.
[2]Controls not tested: Rh — 2; Fy^a — 1; Sec. — 7.
*Only 269 controls tested with anti—S: MS — 71; Ms — 17; MNS — 63; MNs — 64; NS — 15; Ns — 39.
**Other control group used for Kidd (see text).
†D^u (or Rh_0 variant) bloods.

been tested undiluted. Subsequent dilution (1/20) of these fluids caused the disappearance of the non-specific inhibition, but specific inhibition was retained. That is, O salivas continued to inhibit the Ulex extract but failed to inhibit anti-A.

Fresh, repeat saliva specimens obtained from 11 of these individuals did not exhibit the non-specific inhibition of anti-A. It would seem that bacterial flora or contamination with food containing the A antigen would be the most likely explanation of this difficulty.

RESULTS

Table 5 summarizes the serological results in 370 mongols (Plymouth and Coldwater) and 1508 controls. A complete listing of the 370 mongols itemized by institution, sex, birthdate and blood and secretor types is on file in the Department of Human Genetics, and copies of the raw data may be obtained from the authors upon request. Typings for the Kidd locus on the controls, using anti-Jk^a, were considered unreliable. A smaller group, which had been typed with anti-Jk^b, as had been the mongols, was chosen as the control. This group consisted of the parents of children with fatal or near-fatal diseases and was drawn from the greater Detroit area.

It may be noted in table 5 that for the ABO blood group the comparison of O vs. non-O between mongols and controls approaches the 5 per cent level of significance ($\chi^2 = 3.446$); the deficiency of type O mongols is in the direction predicted on the basis of trisomy. However, the magnitude of the shift in O phenotype frequency is only one-third of the maximum which is predicted by the hypothesis of complete linkage of the locus to the centromere and first division nondisjunction. For loci involving more than two alleles, several methods of comparing the mongols and controls are available. Table 5 lists one such comparison for each locus. For the MNS and Rh loci, other comparisons (MN vs. M; MN vs. N; S+ vs. S–; C+ vs. C–; E+ vs. E–) revealed no significant differences between the mongols and the controls.

Table 6 reveals that for three loci, MN, P and Kell, the direction of the shift in blood group frequencies is opposite to that expected on the trisomy hypothesis (column h). The magnitude of the observed shifts is small for all loci tested. Expected trisomic frequencies, derived from the gene frequencies of the control group, indicate that there are no loci which show a good fit with the trisomy hypothesis (column i). Findings in the ABO blood groups require further comment.

A 2 x 2 comparison of A vs. non–A ($\chi^2 = 2.768$; $P = .10 - .05$) suggests a deviation in the direction predicted by trisomy similar to that observed in the O vs. non–O comparison. However, a 2 x 4 comparison of A, B, AB, and O with three degrees of freedom reveals an over-all agreement between the mongols and the controls ($\chi^2 = 3.948$; $P = .30 - .20$). Because of these equivocal findings, a larger sample was obtained to determine whether the fluctuations were due to chance or were, in fact, significant. ABO slide typing of 423 additional mongols (Lapeer and Orient) was undertaken. Before

TABLE 6. COMPARISON BETWEEN EXPECTED AND OBSERVED SHIFTS IN PHENOTYPE FREQUENCIES FOR EIGHT LOCI

Locus	Allele tested	Calculated gene frequency in controls (q)	Observed phenotype frequency in controls (q^2)	Maximum expected phenotype frequency in trisomics (q_3^2)	Observed phenotype frequency in mongols	Maximum shift predicted (e) — (d)	Shift observed (f) — (d)	Chi-square (f) vs. (e)
(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)
ABO	O	.6755	.4562	.3082	.4098	—.1480	—.0464	15.512
Rh	d	.4121	.1698	.0700	.1514	—.0998	—.0184	37.614
MN	N*	.4562*	.2076	.0947	.2216	—.1129	+.0140	69.523
P	p	.4973	.2473	.1230	.2622	—.1243	+.0149	66.426
Kell	k	.9577	.9171	.8783	.9290	—.0507	+.0119	9.156
Duffy	$Fy^a(-)$	.5806	.3371	.1957	.3000	—.1414	—.0371	21.068
Kidd	$Jk^b(-)$	.5906	.3488	.2060	.3321	—.1261	—.0167	27.239
Secretor	se	.4822	.2325	.1121	.2316	—.1204	—.0009	52.661

*Direct gene count.

TABLE 7. ABO TYPINGS OF 793 MONGOLS AT FOUR INSTITUTIONS COMPARED TO CONTROLS. PERCENTAGE FREQUENCIES ARE GIVEN IN PARENTHESES

Institution	A	B	AB	O	Total
Plymouth	53 (46.5)	17 (14.9)	2 (1.8)	42 (36.8)	114
Coldwater	117 (45.7)	22 (8.6)	10 (3.9)	107 (41.8)	256
Lapeer	134 (43.4)	35 (11.3)	11 (3.6)	129 (41.7)	309
Orient	45 (39.5)	20 (17.5)	2 (1.8)	47 (41.2)	114
Total mongols	349 (44.0)	94 (11.9)	25 (3.2)	325 (41.0)	793
Controls	621 (41.2)	144 (9.5)	55 (3.7)	688 (45.6)	1508

O vs. non-O comparisons*

	χ^2	D.F.	P
Total	6.931	4	
Heterogeneity	0.873	3	.90—.80
Meaningful	6.058	1	.02—.01

*Method of B. Woolf (1955).

TABLE 8. COMPARISONS OF ABO BLOOD TYPES OF CONTROL POPULATION AND 793 MONGOLS, POOLED FROM FOUR INSTITUTIONS

Comparisons	χ^2	D.F.	P
O vs. non—O	4.540	1	.05—.02
O vs. A	3.353	1	.10—.05
A vs. non—A	1.707	1	.20—.10
B vs. non—B	2.977	1	.10—.05
AB vs. non—AB	0.379	1	.70—.50
A vs. B vs. AB vs. O	6.563	3	.10—.05

adding the data to the original sample, several comparisons were made. The results, by institution, are presented in table 7. The heterogeneity chi-square and the comparison chi-squares of the four subsamples are low; therefore pooling appears justified.

Table 8 lists the comparisons made between the pooled sample of 793 mongols and 1,508 controls for the ABO locus. The deviations are in the direction expected in trisomy (decreased O, increased A, increased B) except for no increase in AB. It should be noted that under a trisomic hypothesis the frequencies of B and AB are expected to show only slight increases which might be difficult to detect in a sample of the size herein reported.

The gene frequencies of the controls and the mongols were estimated by the maximum likelihood method and are given in table 9. The values for the controls fit well for genetic equilibrium in a diploid population ($\chi^2 = 0.115$). When the mongols are tested on the basis of two chromosomes the goodness of fit approaches the 0.05 level of significance ($\chi^2 = 3.640$) while, if they are calculated on a three chromosome hypothesis, there is obviously poor agreement with genetic equilibrium ($\chi^2 = 12.525$). When a chi-square comparison is made between the gene frequencies of the controls and mongols (assuming two chromosomes) and the homogeneity of estimates tested, the most significant source of variation is in the frequency of the *O* allele.

TABLE 9. GENE FREQUENCY ESTIMATES FOR THE ABO LOCUS (CALCULATED BY MR. ATMARAM H. SONI)

Frequency of allele	1508 Controls	793 Mongols	
		2 chromosomes	3 chromosomes
p_A	0.257	0.274	0.193
q_B	0.068	0.079	0.053
r_O	0.675	0.647	0.754

Partitioned χ^2 between controls and mongols (2 chromosomes)

Source	Degrees of freedom	χ^2
Difference in r	1	3.012
Difference in p + q	1	0.393
Total	2	3.405

DISCUSSION

The results obtained in the present study suggest that if the majority of mongols arise by first meiotic nondisjunction, it is quite unlikely that any of the loci tested are linked to the centromere of chromosome 21, with the possible exception of the ABO. Several lines of inquiry may be brought to bear on the interpretation of the ABO data: (1) Do other published studies of mongol blood groups confirm or conflict with the present findings? (2) Do serological studies in families of mongols clarify the issues? (3) What is the information available from translocation families? (4) Are there any critical O x AB matings resulting in an AB mongol child? (5) Is there any evidence that mongolism may arise by second division or somatic nondisjunction?

(1) *Other Mongol Blood Group Studies.* There have been seven other published series of ABO blood groups in Caucasian mongols. In five of these, the frequency of the O blood group was lower than or equal to the frequency of the A blood group, and in these five series the percentage frequency of the O phenotype ranged from 34.1 to 42.3 (table 10). There is a significant excess

TABLE 10. LITERATURE SURVEY OF ABO FREQUENCIES OF MONGOLS

Investigators	Location	A	B	AB	O	Total	A $\geq$ O
Orel, 1927	Austria	21	7	1	15 (34.1)	44	Yes
Manitz, 1932	Germany	14	1	0	11 (42.3)	26	Yes
Penrose, 1932	England	83	14	3	66 (39.8)	166	Yes
Benda & Bixby, 1939	USA	48	12	5	60 (48.0)	125	No
Engler 1949	England	48	9	3	40 (40.0)	100	Yes
Lang Brown, *et al.*, 1953	England	53	9	5	81 (54.7)	148	No
Haeckel, 1954	USA	24	7	4	24 (40.7)	59	Yes
Shaw & Gershowitz, 1962	USA	349	94	25	325 (41.0)	793	Yes

of O in the data of Lang Brown, Lawler and Penrose (1953) which is accounted for by the fact that 51 per cent of the mothers of the mongols were type O. It is curious that the series of Penrose (1932) and of Lang Brown *et al.* (1953) were both taken from mongols residing in the London area and yet differ significantly from each other for the O vs. non-O comparison ($\chi^2 = 7.043$; $p < .01$).

(2) *Blood Groups of Parents of Mongols.* Trisomy for a blood group locus might be suggested if it could be shown that the mongols more often possess an antigen derived from their mothers than from their fathers. Data from the family studies of Lang Brown et al. (1953) show that among 131 mother-mongol pairs there were 17 mongols who did not possess an A or B antigen present in the mother, while there were 32 such cases among 119 father-mongol pairs. Penrose (1957) has analyzed these results by comparing similarities of blood group antigens in mothers and mongols and fathers and mongols, without regard to specific mating pairs. For the ABO blood group, the mongols resemble their mothers much more frequently than expected ($\chi^2 = 11.05$; $P < .01$) while the father-mongol blood types agree almost exactly as expected. Penrose points out that these results are probably not explained by maternal-fetal incompatibility, even though mongols are frequently "last-born" of older mothers, because incompatible families have stopped reproduction before mongolism is a serious risk and are not likely to be represented. The validity of his reasoning is borne out by the results of 60 families typed in this laboratory. ABO blood types were inspected when there was at least a ten-year age span in the mother between first-and last-born children regardless of family size. Twenty-five of the first-born children differed from the father in ABO type, whereas 26 of the last-born children did so. Further analysis of the British family data show that for the matings of 22 O-male x A or B females, 6 type O mongols were produced, whereas in 28 reciprocal matings, 18 type O mongols resulted ($\chi^2 = 6.76$; $P < .01$). These results are compatible with the hypothesis of ABO trisomy in mongolism if it is assumed that the exceptional cases — when the mongol lacks an antigen present in the mother — result from crossing-over, second meiotic or post-fertilization mitotic nondisjunction.

(3) *Blood Groups of Segregating Translocation Families.* Mongol families in which a translocation chromosome is segregating shed additional light on linkage systems. If a carrier parent possesses an antigen which is lacking in the mongol, the locus for the antigen must be segregating independently of the centromere of the translocation chromosome, or a crossover must have taken place. Here the timing of the disjunctional error is not under consideration. Shaw (1962) has reported a kindred of six translocation mongols in four sibships. In this family three mother-mongol pairs have been blood-typed. A fourth translocation mother-mongol pair in another family has also been investigated (Shaw, in press). Atkins *et al.* (1962) reported the blood groups of three translocation mongol sibs and their parents in which the mother carried the translocation. Penrose and Delhanty (1961) reported blood types in two mother-mongol translocation pairs. Table 11 summarizes the blood group data in nine translocation mother-mongol pairs. Of these nine pairs, three mothers were type O and one was type A_2, thus yielding no information, while four mongols possessed the A or B antigen present in the mother. There is one "exception" for the ABO locus. If this locus is on the mongol chromosome, this "exceptional" mongol must represent a crossover. The MN and Rh loci may reasonably be excluded from chromosome 21 on the basis of these

TABLE 11. SEROLOGICAL RESULTS IN NINE TRANSLOCATION MOTHER-MONGOL PAIRS. MOTHER'S TYPE IS GIVEN FIRST IN EACH PAIRED GROUPING. ALL TRANSLOCATIONS INVOLVE A CHROMOSOME OF GROUP 13-14-15 WITH 21, EXCEPT PAIR NO. 4 WHICH IS A 21/22 TRANSLOCATION

Pair	ABO	MNSs	Rh	P	Kell	Duffy	Kidd	Reference
1	A_2 — A_1	Ms/Ns — MS/Ms*	R_1r — R_1r	P+ — P—*	kk — kk	Fy^a— — Fy^a+	Jk^b— —Jk^b—	Shaw (1962).
2	B — B	MS/Ns — Ns/Ns*	rr — rr	P+ — P+	kk — kk	Fy^a— — Fy^a—	Jk^b+ — Jk^b+	"
3	O — B	MS/Ns — Ns/Ns*	R_1r — r"r*	P+ — P+	kk — kk	Fy^a+ — Fy^a+	Jk^b+ — Jk^b+	"
4	A_1 — A_1B	Ms/Ns — Ns/Ns*	R_1R_1 — R_1r	P— — P—	kk — kk	Fy^a+ — Fy^a—*	Jk^b+ — Jk^b+	Shaw (in press).
5	A_1 — A_1	Ms/Ns — NS/Ns*	R_2r — R_1R_2	NT	kk — kk	Fy^a+ — Fy^a—*	NT	Atkins, *et al.* (1962).
6	A_1 — O*	Ms/Ns — Ms/Ms*	R_2r — R_1R_2	NT	kk — kk	Fy^a+ — Fy^a+	NT	"
7	A_1 — A_1	Ms/Ns — Ns/Ns*	R_2r — R_1r*	NT	kk — kk	Fy^a+ — Fy^a+	NT	"
8	O — O	MS/Ms— Ms/Ns*	R_2R_2 — R_2r	P+ — P+	kk — NT	Fy^a+ — NT	NT	Penrose & Delhanty (1961).
9	O — O	MS/Ms— MS/NS*	R_2R_2 — R_2r	P+ — P+	kk — NT	Fy^a+ — NT	NT	"

NT= not tested.
*An antigen is present in carrier mother and absent in translocation mongol.

data, while information on the P, Kell, Duffy and Kidd loci is insufficient to be informative. Further data are needed for this type of linkage analysis, since it would appear to be the most promising approach.

(4) *Exceptional Inheritance Producing an AB Mongol.* The most critical evidence for linkage would be the production of an AB mongol from a mating of O — male x AB — female. Less than two per cent of all matings in the general population are of this type. In a search for such evidence the parents of seven AB mongols were investigated. The mother was AB in only one instance, but her husband was dead. In the series of Lang Brown et al. (1953) one AB mongol had an AB mother and an A father. Two AB mothers (fathers not tested) had one A and one B mongol.

A type O child born to an AB mother may be due to monosomy (with nondisjunction occurring in the mother), a deletion, or homozygosity for one or two pairs of modifiers. The grossly malformed type O child of an AB mother reported by Haselhorst and Lauer (1930, 1931) may have been monosomic for chromosome 21 (if the ABO locus is, indeed, on that chromosome), but it is not believed that monosomics are viable, since none have been found in translocation families.

(5) *Mongolism Arising by Second Division or Somatic Nondisjunction.* Several references are cited below which suggest that second-division nondisjunction and mitotic nondisjunction may occur in man. Since mongolism is correlated with increasing maternal age, it is probable that maternal nondisjunction is more often responsible than paternal nondisjunction. Meiotic prophase in the mammalian female begins in the embryonic ovary and stops after the diplotene stage has been reached, i.e., after synapsis and crossing-over have occurred (Slizynski, 1961; Ohno et al., 1961). Then the primary oocyte lies dormant in the interphase-like dictyotene stage until the time of ovulation. The second meiotic division is presumably not completed until after fertilization. Desynapsis during this long latent period or nondisjunction in either the first or second meiotic division of the aged ovum could account for trisomy. Alternatively, homologous pairing may be prevented more frequently among the acrocentric chromosomes if nucleolar remnants remain on the heterochromatic short arms, and trisomy could arise by nonconjunction rather than nondisjunction. Lennox (1961) has reviewed data on color blindness in Klinefelter's syndrome and cites three cases of which two derived from a carrier mother and normal father. He suggested that second-division nondisjunction is the simplest explanation. However, crossing-over between the centromere of the X chromosome and the color blind locus cannot be excluded. Cooper et al. (1962) have suggested that a significant proportion of human sex chromosome aneuploids owe their origin to post-fertilization mitotic nondisjunction.

21-trisomy/normal mosaicism has been reported by Clarke et al. (1961) in a female with clinical features of mongolism. It is not possible to determine whether the zygote was diploid or trisomic for 21, but a disjunctional error or chromosome loss must have occurred in mitosis. Fitzgerald and Lycette (1961) have reported a mongoloid triple mosaic (di-21/tri-21/tetra-21) which is interpreted as a mitotic nondisjunction in an early cleavage of a zygote which was already trisomic for this chromosome.

Finally, the possibility of blood group-disease association should be mentioned as an alternate interpretation for the ABO data reported here. In the established cases of such association (see review by Roberts, 1959) an excess of one phenotype is found with a deficiency of the others; in the present study there is an increase in A and B and a decrease in O. More data, particularly family studies, are needed to clarify the issues raised.

If linkage associations are discovered by translocation family studies, population surveys such as this will serve to yield information on the frequency of the various mechanisms giving rise to nondisjunction in man.

SUMMARY

Blood and saliva typings of 370 mongols indicate that the MNS, P, Kell, Duffy, Kidd and ABH secretor loci are not closely linked to the centromere of chromosome 21. Only the ABO locus yields results which are suggestive of linkage: Among a total of 793 mongols typed for ABO there was a deficiency of group O ($P = .05 - .02$) and an excess of groups A and B. The possible causes of this deviation may be: (1) linkage of the ABO locus with chromosome 21 but at some distance from the centromere, (2) blood group-disease association, (3) non-randomness of the blood group frequencies among the parents of the mongols, and (4) chance. Various lines of inquiry which may yield more information are discussed.

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