Am J Hum Genet 29:431–447, 1977

Chromosome Polymorphism and Twin Zygosity

DANIEL L. VAN DYKE,[1] CATHERINE G. PALMER,[2] WALTER E. NANCE,[3]
AND PAO-LO YU[2]

INTRODUCTION

Since the early 1960s normal variation of human chromosome morphology has been documented at a prodigious rate. Banding techniques [1, 2] have demonstrated that a number of chromosome regions are polymorphic and that specific variants differ in frequency among normal populations [1–15]. Although heteromorphism at these "variable regions" is not generally believed to have an observable effect on phenotype [3], differences in the frequency of certain variants between normal and abnormal groups continue to be reported [4, 16–19]. Even though variants have been employed as markers for linkage studies, segregation analysis, paternity exclusion, maternal-fetal comparisons, and for tracing meiotic errors, there is still some uncertainty as to their stability [20–22].

The present study was designed to evaluate the heritability and stability of the variable regions and to estimate the frequency of variants in twins. A central goal was to determine by detailed chromosome studies of monozygotic (MZ) and dizygotic (DZ) twins whether each person's individuality might be reflected in a unique distribution of chromosome variants (cf. [23]).

METHODS

Forty like-sexed pairs of Caucasian twins (23 MZ and 17 DZ) were obtained from a panel at the Indiana University School of Medicine, Department of Medical Genetics. About half were adolescents and pre-adolescents; the remaining pairs were college students. In addition, a set of male quadruplets and their parents were studied. Zygosity was determined by genotyping for the loci of blood groups ABO, Rh, MNS, Fy, P, JK and serum proteins Hp and PGM. The probability of misclassifying a set of like-sexed DZ twins as MZ was less than .01 [24]. Each DZ pair was discordant in at least two loci.

Peripheral blood was cultured for chromosome analysis according to standardized procedures. The method of G-banding was a modification of the technique of Summer et al. [25] and

Received November 24, 1976; revised May 12, 1977.

This work was supported by the John A. Hartford Foundation, genetic training grant GM 1056 from the U.S. Public Health Service, and Public Health Service grant GM 21054 of the Indiana University Human Genetics Center.

[1] Henry Ford Hospital, 2799 W. Grand Boulevard, Detroit, Michigan, 48202. Address reprint requests to D. L. Van Dyke.

[2] Department of Medical Genetics, Indiana University School of Medicine, Indianapolis, Indiana.

[3] Department of Medical Genetics, Medical College of Virginia, Richmond, Virginia.

© 1977 by the American Society of Human Genetics. All rights reserved.

Q-banding, the technique of Lin and Uchida [26]. The sequential G- to C-banding method was modified from Lubs et al. [27], using 12XSSC buffered with pH 7.0 McIlvaine's buffer (50:1). The destained slide was warmed on a hot plate at 60°C for 18 min, treated in 0.2 N HCl for 15 min, and placed in three distilled water rinses (5 seconds, 1 min, and 5 min). It was then incubated for 3 hr in 60°C buffered 12XSSC, rinsed again in distilled water, stained in 2:48 Harleco Giemsa in pH 6.8 phosphate buffer, rinsed in pH 6.8 phosphate buffer, and blotted dry. Scoring of G-band and Q-band heteromorphism was done by microscope and occasionally confirmed in photographs made with Kodak High Contrast Copy Film.

Q-band variants were scored as recommended by the Paris Conference [1, 2]. Nine variable regions were scored after Q-banding (the paracentromeric regions of chromosomes 3, 4, 13, and 22; and the short arm/satellite regions of all five pairs of acrocentrics). Variations in morphology, as well as intensity, were observed. For example, short arms or satellites frequently differed in size between homologs, and occasionally a short arm plus satellites ("ps") variant was seen.

Sequentially G- to C-banded chromosomes were compared to see if the C-band variants of chromosomes 1, 9, and 16, which are common in Caucasians, were distinguishable by both techniques. Numerical scores of 2–9, from smallest to largest, were assigned to a reference set of C-band variants used in our laboratories [28]. Three fairly common variants on chromosomes 1, 9, and 16 were observed in this study and were assigned scores of 4, 5, and 6. These are all within the range of "normal" as defined by Craig-Holmes et al. [29]. From five to 15 pairs of each of the homologous chromosomes were scored in each individual by an observer (D. V. D.) who was unaware of the true zygosity.

RESULTS

Comparison of G- to C-Banding for Scoring Paracentromeric Heterochromatin Variants

Variants for the 1qh region were seen in the homozygous and the heterozygous state (fig. 1). Variant 5 was characterized by a single dark band in the proximal long arm variable region. Variant 4 had a narrower dark-staining band, and variant 6 had two narrow bands, separated in most cells by a narrow light-staining band. Two examples of each 1qh variant are shown sequentially G- and C-banded in figure 2. The sequential technique gives a 1qh region which is similar to that seen after NaOH C-banding [28]. Either technique offers fine detail; the C-band regions seem to stain darkest after the NaOH C-banding method.

In chromosomes 9 and 16 (fig. 3), the most commonly seen variants were labeled variant 5, with a smaller variant 4, and a larger variant 6. An "inversion" (inv) was also seen on chromosome 9 in which the variable region appeared in the short arm. The light staining 9qh region seen with G-banding was dark-staining after C-banding (fig. 4), but the dark centromere seen after G-banding appeared to be in the C-band positive region as well. The centromere itself did not vary in size among individuals and was distinguishable only in the best preparations.

Repeated Q-Band Scoring

The Q-band scoring was repeated on six MZ twin pairs without knowledge of the original scores; the same slides but different cells were scored to evaluate the precision of the observer on separate occasions. Two twin pairs had the same score both times, but in one MZ pair, the 22c region of one homolog was consistently scored as pale, and the other homolog was first scored as intense fluorescence but subsequently as

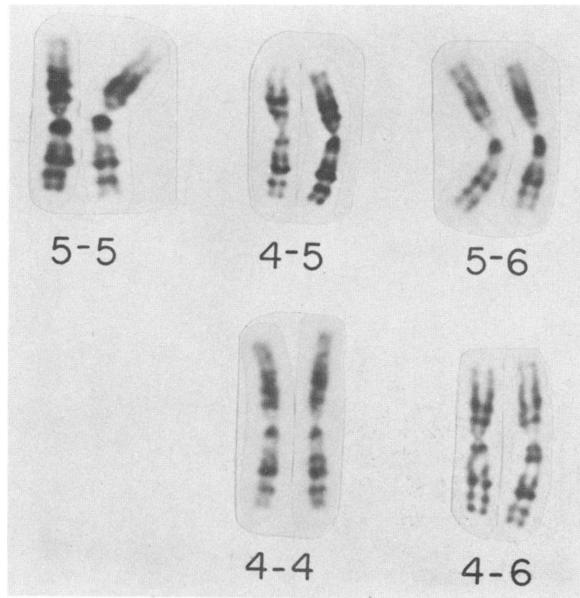

FIG. 1.—Chromosome 1qh variants visualized with G-banding. Homologous pairs of chromosome no. 1 are shown. See text for details.

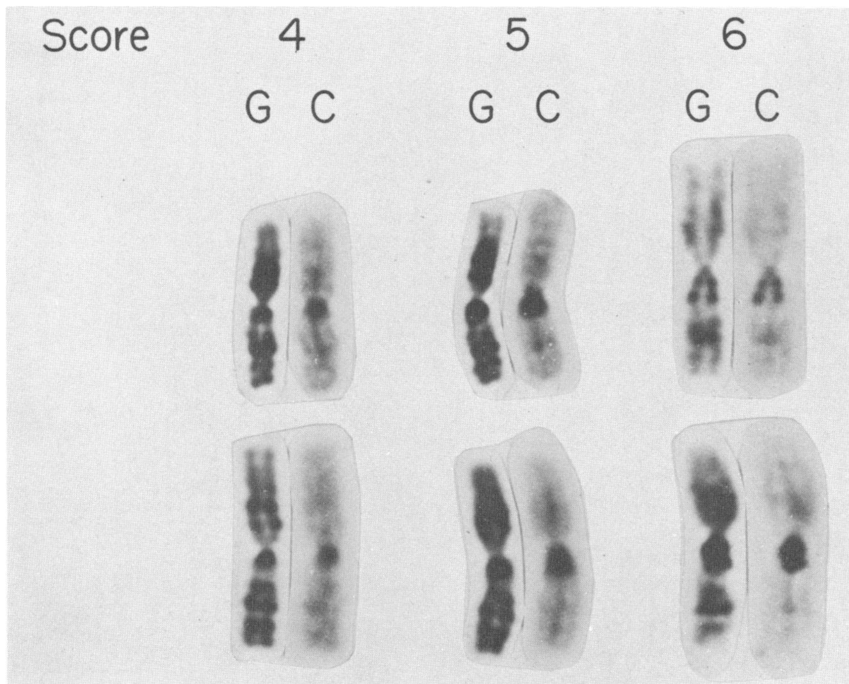

FIG. 2.—Sequential G- to C-banding of 1qh variants. Two examples of each variant are given to illustrate that the two techniques are comparable in extent of material stained in the paracentromeric variable region.

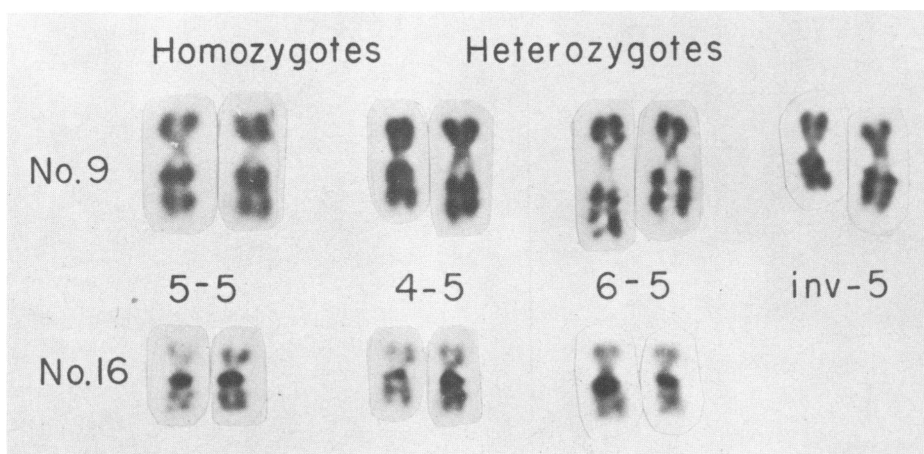

FIG. 3.—Chromosome 9qh and 16qh variants visualized with G-banding. Homologous pairs are shown. Homozygotes (5-5) and heterozygotes are illustrated.

medium. In another MZ pair, the scores of both 22c regions were changed from pale to intense in both siblings. This represented the only case of a two-step change in score. In the same twins, an intense 4c region had been scored medium on the first scoring. Other scoring discrepancies involved chromosomes 4, 14, and 15. The scoring of the

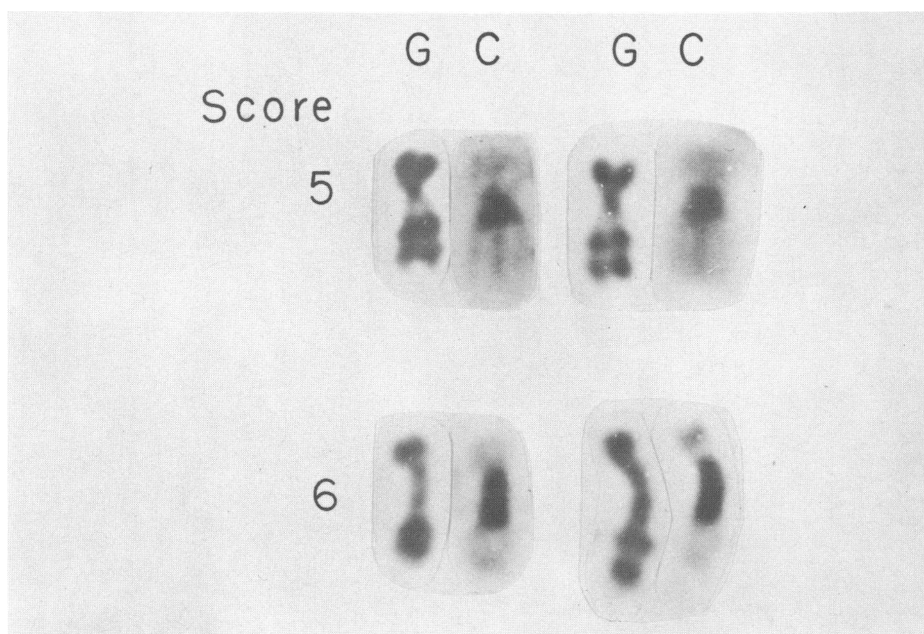

FIG. 4.—Sequential G- to C-banding of 9qh variants. Two examples of each variant (5 and 6) are given to compare the extent of dark-stained material. See text for details.

22c region showed the greatest variability. Of 216 opportunities for error in scoring (two subjects in each of six twin sets, nine variable regions, and two homologous chromosomes), there were two two-step and eight one-step scoring changes.

Frequencies of G- and Q-Banding Variants

The variant frequencies have been estimated using Cotterman's weighting system for family data on genes without dominance [30]. By his criteria, each MZ twin pair counted as a single individual, while each member of a DZ pair was assigned a weight of two-thirds of one unrelated individual. The parents of the quadruplets were counted as two unrelated individuals, and the quadruplets themselves were excluded from the estimate. The total sample size after weighting (i.e., sum of the weights) was 47.67 individuals or a total of 95.33 chromosomes.

The frequency of 1qh, 9qh, and 16qh variants scored with G-banding are given in table 1. The five levels of Q-band fluorescence were pooled to two levels to facilitate comparison of our data to that in the literature; "light" consisted of negative, pale, and medium variants, and "bright" included intense and brilliant variants (table 2). The observed frequency of homozygotes and heterozygotes for each variant was compared to Hardy-Weinberg expectation, and good agreement was found between observed and expected phenotype distributions for every marker.

Distinguishing Zygosity in Twins and a Set of Quadruplets

The scores of the male quadruplets and their parents are given in table 3 to

TABLE 1

FREQUENCY ESTIMATES (± SE) FOR G-BAND VARIANTS

Variable Region	4	5	6	inv
1qh	.31 ± .07	.60 ± .07	.09 ± .04	...
9qh	.03 ± .02	.83 ± .05	.13 ± .05	.01 ± .01
16qh	.11 ± .05	.88 ± .05	.01 ± .01	...

TABLE 2

FREQUENCY ESTIMATES (± SE) FOR Q-BAND BRIGHTNESS VARIANTS

Variable Region	Light	Bright
3c	.32 ± .07	.68 ± .07
4c	.73 ± .06	.27 ± .06
13c	.12 ± .05	.88 ± .05
22c	.51 ± .07	.49 ± .07
13p	.97 ± .02	.03 ± .02
14p	.95 ± .03	.05 ± .03
15p	.92 ± .04	.08 ± .04
21p	.92 ± .04	.08 ± .04
22p	.93 ± .04	.07 ± .04

TABLE 3

CHROMOSOME VARIANT SCORES FOR 37 MZ AND 20 DZ TWIN SETS AND THE QUADRUPLETS AND THEIR PARENTS

Variable Region	1qh	9qh	16qh	3c	4c	13c	22c	13p	14p	15p	21p	22p
Twin 37a	4-5	5-6	5-5	5-5	3-3	3-5	3-3	2-2	2-2	2-2	2-3	2-2
Twin 37b	4-5	5-6	5-5	5-5	3-3	3-5	3-3	2-2	2-2	2-2	2-3	2-2
Twin 20a	4-4	5-5	5-5	3-3	3-5	5-5	2-2	2-2	2-2	2-2	2-2	1-4
Twin 20b	4-5	5-5	5-5	3-5	3-3	5-5	2-2	1-2	2-2	2-2	2-2	1-2
Discordance	*	...	...	†	†	...	...	*	...	...	...	†
Quad A	4-6	5-5	5-5	4-5	3-5	5-5	2-4	2-2	2-2	2-3	3-5	2-4
Quad B, C, D	4-4	5-5	5-5	3-5	3-5	5-5	4-4	2-2	2-2	2-3	2-5	2-2
Discordance	†	...	...	*	...	...	†	...	...	...	*	†
Mother	4-4	5-5	5-5	4-5	3-5	5-5	4-4	2-2	2-2	2p4s-2	2-5	2-2
Father	4-6	5-5	5-5	3-5	3-3	5-5	2-4	2-2	2-2	2-3	2-3	2-4

NOTE.—The numerical scores are those recommended by the standing committee of the Paris Conference [2].
* Marginal discordance (one step in the 5-step Paris schedule).
† Non-marginal discordance.

demonstrate how the quadruplets were established as asymmetrically dizygous. Quadruplets B, C, and D had no discordances among them, whereas quadruplet A was distinct from B, C, and D on the basis of five karyotypic differences. This agreed with the genotyping data which indicated that sibs B, C, and D were monozygotic and that sib A developed from a second zygote.

A comparison of the variant scores among the quadruplets and their parents agreed with the expected Mendelian segregation pattern. All of the quadruplets had a brilliantly fluorescent 3c, but this variant was not identical by descent for subject A and his monozygous sibs. Subject A inherited his mother's intense 3c, so his brilliant 3c was of paternal origin; the other boys had the paternal medium 3c, so their brilliant 3c must have been of maternal origin.

Twelve variable regions were scored by G- and Q-banding from the metaphase cells of 40 like-sexed twin sets. Ten chromosome pairs carried the 12 variable regions (giving a total of 24 scores for each subject); chromosomes 13 and 22 bear both centromeric and satellite variable regions.

Variant scores of representative MZ and DZ twin sets are given in table 3. There were no differences in score between the members of MZ twin set 37. However, between the members of DZ twin set 20 there were discordances at five polymorphic regions (1qh, 3c, 4c, 13p, and 22p).

From the total of 552 comparisons made between 23 MZ twins (23 twin pairs, 12 variable regions, and two homologous chromosomes), no discordances between MZ twins were scored at any variable region. Of 17 DZ twin pairs scored, 12 had discordant chromosome scores. Chromosomal discordances ranged from three Q-banding (one nonmarginal, two marginal) discordances between the twins of pair number 3, to two G-banding plus five Q-banding (only one marginal) discordances between the members of twin pair 39 (table 4). All 12 of the twin pairs with discordant karyotypes had at least one nonmarginal Q-banding discordance. Nine of the 12 had one or two G-banding discordances.

Although five DZ twin pairs were scored as having no karyotypic differences, a possibility of differences was noted in three cases; these questionable differences were thought to be technical artifacts. For example, both members of DZ twin set 9 were scored as heterozygous (medium/pale) on chromosome 21 with Q-banding. However, the pale homolog appeared more pale in subject 9a than in 9b. Furthermore G-banding scores of satellite morphology and size suggested that in twin 9a, but not in twin 9b, one chromosome 21 had satellites smaller than those in its homolog (Van Dyke et al., unpublished data). Similarly, another DZ twin pair may have been discordant at region 15p and another at 9qh.

DISCUSSION

Comparison of Scoring Techniques for Chromosomes 1, 9, and 16

Hauge et al. [31] demonstrated the applicability of Q-banding for scoring variants of chromosomes 1, 9, and 16; however, McKenzie and Lubs [5] missed 50% of these using Q-banding alone. R-banding, especially the acridine-orange fluorescent R-banding [32–34], is gaining in popularity. Verma and Lubs [33–34] find the

TABLE 4

NO. OF G-BANDING AND Q-BANDING DISCORDANCES WITHIN DZ PAIRS

BANDS	DZ TWIN PAIR IDENTIFICATION NO.*												
	3	4	7	13	14	20	26	27	28	35	39	40	Quadruplets
G-bands	0	1	0	0	1	1	2	1	1	1	2	1	1
Q-bands†	1	1	2	4	3	3	3	4	2	2	4	2	2
Marginal Q-bands	2	1	4	0	1	1	0	0	1	1	1	1	2
Total	3	3	6	4	5	5	5	5	4	4	7	4	5

* DZ twin pairs 9, 23, 24, 31, and 34 were scored as having no discordances.
† Does not include marginal Q-band discordances.

technique useful because the light-staining variable bands of chromosomes 1, 9, and 16 are bordered by sharply defined bright bands. Although C-banding remains a viable alternative for polymorphism scoring, not all chromosomes are identifiable by C-banding alone, and sequential techniques (Q- or G-banding to C-banding) are too time-consuming for routine use. Craig-Holmes et al. [29] showed that even without sequential techniques additional polymorphisms were observable within all groups except C6-8, 10-12.

The advantage of G-banding is that all chromosomes are unambiguously identifiable, and chromosome 1, 9, and 16 heteromorphism can be detected. Thus, G-banding can be used to score paracentromeric polymorphism in large clinical populations with little additional effort.

Precision of Scoring Q-Band Brightness Polymorphism

Replicate analysis in this study illustrated the difficulty in achieving reproducible scoring of Q-band brightness variants. Marginal discrepancies in score (one-step changes in score) were not rare, and a nonmarginal discrepancy was encountered once. These changes were seen mostly in the 22c and 4c regions which are small and difficult to visualize. Madan and Bobrow [35] and McKenzie and Lubs [5] have experienced similar problems with other variable regions, emphasizing the need for high quality preparations and technical expertise.

Such imprecision can lead to two kinds of error that are analogous to type I and type II errors in statistical analysis [36]. In a twin study, qualitative scoring could and probably has led to a failure to identify differences which were in fact present (type II error) because of a conservative approach to the scoring. If a difference between twins was not striking, then it might have been scored as "no difference"; conversely, in a study where one actively seeks differences between subjects or has an a priori expectation of their existence, differences could be scored where no inherited differences existed (type I error). For example, differences are sought to identify fetal cells in amniotic cultures. Using Q-banding, Hauge et al. [31] scored karyotypic variants on 10 chromosomes in 50 mother-fetus pairs and observed six or more differences between mother and fetus in 28 pairs (56%), with no pair scoring fewer than two differences. In contrast, we observed six or more differences between twins in only two of seventeen DZ twin pairs (12%). In the case of fetal-maternal comparisons, any suggestion of karyotypic differences might be too readily accepted, since one rather expects the maternal lymphocyte karyotype to differ from the presumed fetal karyotype, whereas the converse is true in twin comparisons.

Population Frequencies of Variants

Although we recognize the difficulties in comparing our results with those of other investigations, the estimated frequencies of variants seen in the present study were similar to those obtained in other Caucasian populations. Estimates of 1qh+ frequencies range from .01 to .08 in the literature (table 5). The frequency of variant 6 here for 1qh (.09 ± .04) is similar to the frequency (.08) of "large" variants (1½ times the length of 21q) reported by Muller et al. [6], suggesting that possible inclusion of our variant 6 in their "large" classification may explain their comparatively higher

TABLE 5

POPULATION FREQUENCY ESTIMATES FOR CENTROMERE (C-BAND POSITIVE) REGION VARIANTS

SAMPLE	SOURCE	CHROMOSOME 1			CHROMOSOME 9			CHROMOSOME 16			SAMPLE SIZE*	REFERENCE
		+	−	inv	+	−	inv	+	−	inv		
Caucasian	Texas	.02	.10	0	.05	.08	0	0	.18	0	40	[29]
Dutch	Netherlands	.02	0	0	.14	0	0	.01	0	0	442	[6]
90% Caucasian	Colorado	.04	.04	0	.07	.04	.04	.05	.12	0	154	[5]
Mixed, 67% Caucasian	New York	.08	.01	.02	.08	.01	.11	.06	.24	.01	>700	[7]
Mostly Caucasian	Indiana	.01	.09	.02	...	...	...	...	...	...	148	[37]
		...	...	...	.07	.01	0	...	...	...	86	
		...	...	...	...	...	...	.06	.01	0	105	
Oriental	Indiana	.04	.24	.07	.06	.08	.31	.09	.18	0	98	[38]
Caucasian	Indiana	0	0	0	.13	.03	.01	.01	.11	0	94	Present study

* Sample size = no. of chromosomes examined; twice the number of subjects in most cases.

estimate. The variants scored 4 and 5 here may represent two levels of a continuous distribution in the quantity of heterochromatin, whereas the variant scored 6 may be a discontinuous variant arising from a simple duplication of the heterochromatin of variant 4 (cf. [39]).

The observed frequencies of 9qh− and 9qh+ (scored here as variant 4 and 6, respectively) agree with many of the estimates given in the literature (table 5). The frequency of inv(9) seen here agrees with the data of McKenzie and Lubs [5]; the higher estimate obtained by Muller et al. [6] may be a result of the large number of non-Caucasians (33%) in their sample. Even a few Orientals could have increased their proportion of 9qh inversions, since Park and Antley [38, 40] found "position" variants in a larger proportion of the no. 9 chromosomes in Orientals.

Frequency estimates for 16qh− in the literature cover a wide range (table 5); our results fall between the extremes and agree with those of McKenzie and Lubs [5]. The frequency of 16qh+ variants is somewhat lower here than many of the estimates given in the literature. Only Muller et al. [6] have observed the rare inv(16) variant.

For the fluorescence intensity variants of 3c, 4c, 13c, and 22c, the estimates obtained here agree with the literature (table 6) except for 22c, where we observed a somewhat higher frequency. The 22c variable region is probably the smallest being scored; consequently, it is not too surprising that estimates of bright variant frequency are so divergent. Estimates for the frequency of bright variants at 3c and 13c are quite similar among most laboratories, but frequency estimates for bright 4c variants range widely, probably again reflecting the difficulty of scoring fluorescence in the smaller variable regions.

The frequencies of bright satellites and short arm variants found here are difficult to compare with those of other studies because different criteria were used to define the precise region to be scored and whether size and morphology were to be scored separately from fluorescence intensity. The criteria used in the studies by McKenzie and Lubs [5] and Muller et al. [6] are the most readily comparable to ours (table 7). The frequencies given here for McKenzie and Lubs were obtained by pooling their "intensity variants" and "borderline intensity variants." These pooled frequencies are very similar to ours, as are Muller and Klinger's estimates for D group chromosomes. However, Muller and Klinger reported a much higher frequency of bright variants on both chromosome 21 and 22. In their Caucasian sample, two to three times as many bright 21p variants and over three times as many bright 22p variants were found. McKenzie and Lubs [5] found 2.9 bright Q-band variants per person, whereas we found 3.4. Much of this difference can be accounted for by differences between the 22c region variant frequencies.

The frequency of heterozygotes and homozygotes was not significantly different from Hardy-Weinberg expectations at any variable region, nor did males and females differ in their frequency of variants or distribution of heterozygotes and homozygotes, a finding which contrasts with reports from Mikelsaar et al. [4, 9] and Muller et al. [6]. Decreased heterozygosity of 3c variants in males [9] might have been related to the use of the Y chromosome as a landmark for comparison of brightness levels; because the comparison is unavailable in female metaphases, subtle differences in scoring criteria between the sexes could exist. This may be an important criticism of the Paris

TABLE 6

POPULATION FREQUENCY ESTIMATES FOR CENTROMERE FLUORESCENCE INTENSITY VARIANTS

Sample	Source	Region 3c +	Region 3c −	Region 4c +	Region 4c −	Region 13c +	Region 13c −	Region 22c +	Region 22c −	Sample Size*	Reference
Caucasian	Sweden	.28	.72	...	...	...	...	...	...	92	[41]
Caucasian	Switzerland	.59	.41	...	...	...	...	...	...	100	[42]
Dutch	Netherlands	.48	.52	.03	.97	.50	.50	.22	.78	442	[8]
90% Caucasian	Colorado	.76	.24	.41	.59	.61	.39	.07	.93	154	[5]
Caucasian	New York	.54	.46	.13	.87	.74	.26	.19	.81	444	[6, 7]
Negro	New York	.68	.32	.18	.82	.80	.20	.27	.73	78	
Estonian	Russia	.65	.35	...	...	...	...	...	...	414	[4]
Estonian	Russia	...	...	...	...	.84	.16	...	...	408	
Estonian	Russia	...	...	.28	.72	...	...	...	...	206	
Estonian	Russia	...	...	...	...	...	...	.36	.64	164	
Caucasian	Indiana	.68	.32	.27	.73	.88	.12	.49	.51	94	Present study

NOTE.—Chromosome scores pooled into two groups for convenience: + = "bright" and − = "light" where "bright" = brilliant and intense.

* Sample size = no. of chromosomes; twice the no. of subjects.

TABLE 7

POPULATION FREQUENCY ESTIMATES FOR ACROCENTRIC SHORT ARM REGION FLUORESCENCE INTENSITY VARIANTS

Sample	Source	Region 13p		Region 14p		Region 15p		Region 21p		Region 22p		Sample Size*	Reference
		+	−	+	−	+	−	+	−	+	−		
Caucasian	New York	.10	.90	.09	.91	.07	.93	.16	.84	.24	.76	444	[6, 7]
Negro	New York	.11	.89	.14	.86	.08	.92	.18	.82	.38	.62	78	
90% Caucasian .	Colorado	.04	.96	.11	.89	.08	.92	.05	.95	.07	.93	154	[5]
Caucasian	Indiana	.03	.97	.05	.95	.08	.92	.08	.92	.07	.93	94	Present study

NOTE.—Chromosome scores pooled into two groups for convenience: + = "bright" and − = "light", where "bright" = brilliant and intense.
* Sample size = no. of chromosomes; twice the no. of subjects.

Conference [1] which recommends the use of the brilliant Yq band as a standard of brightness for the identification of other brilliant bands.

Distinguishing Zygosity with Chromosome Polymorphism

Twin zygosity determination by scoring polymorphism on chromosomes 1, 9, and 16 with G-banding and on 3, 4, and the acrocentric chromosomes with Q-banding met with good success. In complete agreement with the genotyping data, chromosome variant scores of a set of quadruplets showed them to be asymmetrically dizygotic. The scoring results of the quadruplets and their parents were consistent with simple Mendelian segregation of the Q-banding and G-banding variants. A few cases of asymmetrically dizygotic quadruplets have been reported [43–45], but this appears to be the first documentation of *like-sexed* asymmetrical dizygotic quadruplets.

Of seventeen pairs of DZ twins, 12 pairs had karyotypic discordances, and five pairs were scored as having identical karyotypes. Each of the pairs scored with different karyotypes had at least three karyotypic discordances (table 4). This suggests that some of the five identical scores may have had less striking differences that were overlooked due to conservatism in scoring.

Within each of the 23 MZ twin pairs there was complete concordance, indicating that chromosome variants are stable and highly heritable. This contrasts with the findings of Craig-Holmes et al. [20] and Sekhon and Sly [22] that alterations in morphology of C-band positive regions occur with some regularity. Thus, it will be of considerable interest to examine further the dynamics of change in variable regions. Variation among somatic cells resulting from age, sexual maturation, or senescence, for example, might not lead to differences between MZ twins.

The Uniqueness of One's Karyotype

To evaluate the uniqueness of the karyotypes encountered, the variant scores of each genetically distinct individual (MZ twin pairs being genetically indistinguishable) were compared to those of every other individual in the study. Among the 61 genetic individuals (23 MZ pairs, 17 DZ pairs, two distinct karyotypes among the quadruplets, and two parents of the quadruplets), there were 56 different karyotypes. The five pairs of individuals with similar karyotypes were the five DZ twin pairs who were scored as having no karyotypic differences. These five DZ twin pairs were the only exceptions to the generalization that every distinct genotype in the study exhibited a different karyotype.

Although five DZ twin pairs were scored as indistinguishable, three of them, as noted previously, may not have had identical karyotypes. A goodness-of-fit chi-square test showed that it would not be surprising to find two of seventeen DZ pairs with truly indistinguishable karyotypes ($\chi^2 = 2.669$; $P > .05$).

The probability that two people taken at random have identical karyotypes was calculated from the variant frequency estimates to be approximately .001. This probability was increased to .04 in the case of sib pairs (or DZ twins).

SUMMARY

To demonstrate the heritability, stability, and frequency of chromosome polymorph-

ism and to assess the value of chromosome studies in the determination of twin zygosity, Q- and G-band chromosome studies were performed on blood samples from MZ and like-sexed DZ twin pairs, and one set of male quadruplets. All of the karyotypic examinations were performed without knowledge of true zygosity.

The quadruplets were shown to be asymmetrically dizygotic. The scoring results of the quadruplets and their parents were consistent with simple Mendelian segregation of the variants. There were 46 sib-sib comparisons available: 23 MZ twin pairs, 17 DZ twin pairs, and among the quadruplets, three MZ and three DZ comparisons. No differences were found between any of the 26 MZ pairs at any of 12 variable regions. Between the pairs shown to be dizygous on genotyping, 15 of 20 had three or more karyotypic discordances. Thus about 90% of the twin and quadruplet pairs were assigned their correct zygosity solely by comparisons of chromosome variant scores, with complete concordance of the MZ pairs' scores.

The karyotype of a genetically distinct individual is virtually unique to himself. Two people taken at random have about one chance in 1,000 of sharing identical karyotypes using scoring criteria of this study. Furthermore, only one pair of sibs in 25 would be expected to have identical karyotypes.

Chromosome variants scored here included 1qh, 9qh, and 16qh scored with G-banding; and variable regions of 3, 4, 13, and 22 and the acrocentric satellites scored with Q-banding. Variants seen at these regions are heritable and stable, since differences were found between DZ twins only, with complete concordance between MZ twins.

ACKNOWLEDGMENTS

We would like to thank Drs. Richard Bockrath, A. Donald Merritt, Paul S. Ing, and Lester Weiss for encouragement and helpful comments.

REFERENCES

1. PARIS CONFERENCE (1971): Standardization in human cytogenetics. *Birth Defects: Orig Art Ser* 8(7), New York, The National Foundation, 1972
2. PARIS CONFERENCE (1971): Standardization in human cytogenetics. *Birth Defects: Orig Art Ser* (suppl.)11(9), New York, The National Foundation, 1975
3. LUBS HA, RUDDLE FH: Applications of quantitative karyotypy to chromosome variation, in *Human Population Cytogenetics*, edited by JACOBS PA, PRICE WH, LAW P, Baltimore, Williams and Wilkins, 1970, pp 119–142
4. MIKELSAAR A-VN, KAOSAAR ME, TUUR SJ, VIIKMAA MH, TALVIK TA, LAATS J: Human karyotype polymorphism. III. Routine and fluorescence microscopic investigation of chromosomes in normal adults and mentally retarded children. *Humangenetik* 26:1–23, 1975
5. MCKENZIE WH, LUBS HA: Human Q and C chromosomal variations: distribution and incidence. *Cytogenet Cell Genet* 14:97–115, 1975
6. MULLER H, KLINGER HP, GLASSER M: Chromosome polymorphism in a human newborn population. II. Potentials of polymorphic chromosome variants for characterizing the idiogram of an individual. *Cytogenet Cell Genet* 15:239–255, 1975
7. MULLER H, KLINGER HP: Chromosome polymorphism in a human newborn population, in *Chromosomes Today*, vol. 5, edited by PEARSON PL, LEWIS KR, Jerusalem, John Wiley, 1975, pp 249–260
8. GERAEDTS JPM, PEARSON PL: Fluorescent chromosome polymorphisms: frequencies and segregation in a Dutch population. *Clin Genet* 6:247–257, 1974

9. MIKELSAAR A-VN, VIIKMAA MH, TUUR SJ, KAOSAAR ME: Human karyotype polymorphism. II. The distribution of individuals according to the presence of brilliant bands in chromosomes 3, 4, and 13 in a normal adult population. *Humangenetik* 23:59–63, 1975
10. MIGEON BR: Familial variant autosomes: new human cytogenetic markers. *Bull Johns Hopkins Hosp* 116:396–402, 1965
11. MILLER OJ, MUKHERJEE BB, BREG WR: I. Normal variations in human karyotype. *Trans NY Acad Sci* 24:372–382, 1962
12. CASPERSSON T, ZECH L, JOHANSSON C, LINDSTEN J, HULTEN M: Fluorescent staining of heteropycnotic chromosome regions in human interphase nuclei. *Exp Cell Res* 61:240–474, 1970
13. CASPERSSON T, HULTEN M, LINDSTEN J, ZECH L: Distinction between extra G-like chromosomes by quinacrine mustard fluorescence analysis. *Exp Cell Res* 63:240–243, 1970
14. CASPERSSON T, LOMAKKA G, ZECH L: The 24 fluorescence patterns of the human metaphase chromosomes. *Hereditas* 67:89–103, 1971
15. MOSCETTI G, PETRIAGGI M, BARBAROSSA CG, TIBERT S: Fluorescence staining method for the morphological and structural study of human chromosomes. *Humangenetik* 12:56–58, 1971
16. KUNZE J, MAU G: A_1 and C_9 marker chromosomes in children with combined minor and major malformations. *Lancet* 1:273, 1975
17. LUBS H, PATIL S, KIMBERLING W, BROWN J, COHEN M, GERALD P, HECHT F, MOORHEAD P, MYRIANTHOPOULOS N, SUMMITT R: Correlations between low IQ, race, and variations in Q and C banding (abstr.). *Am J Hum Genet* 25;47A, 1973
18. NIELSEN J, FRIEDRICH U, HREIDARSSON AB, ZEUTHEN E: Frequency of 9qh+ and risk of chromosome aberrations in the progeny of individuals with 9qh+. *Humangenetik* 21:211–216, 1974
19. CHRISTENSEN KR, NIELSEN J: Incidence of chromosome aberrations in a child psychiatric hospital. *Clin Genet* 5:205–210, 1974
20. CRAIG-HOLMES AP, MOORE FB, SHAW MW: Polymorphism of human C-band heterochromatin. II. Family studies with suggestive evidence for somatic crossing over. *Am J Hum Genet* 27:178–189, 1975
21. HOEHN J, MARTIN GM: Heritable alteration of human constitutive heterochromatin induced by mitomycin C. *Exp Cell Res* 75:275–278, 1972
22. SEKHON GS, SLY WS: Inheritance of Q and C polymorphism (abstr.). *Am J Hum Genet* 27:79A, 1975
23. HECHT F, WYANDT HE, ERBE RW: Revolutionary cytogenetics. *N Engl J Med* 285:1482–1484, 1971
24. GAINES RE, ELSTON RC: On the probability that a twin pair is monozygotic. *Am J Hum Genet* 21:457–465, 1969
25. SUMNER AT, EVANS HJ, BUCKLAND RA: New techniques for distinguishing between human chromosomes. *Nature* [*New Biol*] 232:31–32, 1971
26. LIN CC, UCHIDA IA: Fluorescence banding of chromosomes (Q-band), in *Tissue Culture Methods and Applications,* edited by DRUSE PF JR, PATTERSON MK JR, New York, Academic Press, 1973, pp 778–781
27. LUBS HA, MCKENZIE WH, PATIL SR, MERRIK S: New staining methods for chromosomes. *Methods Cell Biol* 6:345–370, 1973
28. RIVAS ML, CONNEALLY PM, HECHT F, LOVRIEN EW, MAGENIS E, MERRITT AD, MEYERS DA, PALMER CG, WANG L: Linkage relationships of 1qh to Amy, Fy, PGM, and Rh. *Rotterdam Conference (1974): Second International Workshop in Human Gene Mapping. Birth Defects: Orig Art Ser* 11(3), New York, The National Foundation 1975, pp 239–247
29. CRAIG-HOLMES AP, MOORE FB, SHAW MW: Polymorphism of human C-band heterochromatin. I. Frequency of variants. *Am J Hum Genet* 25:181–192, 1973
30. COTTERMAN CW: A weighting system for the estimation of gene frequencies from family records. *Contrib Lab Vert Biol* 33:1–21, 1947

31. HAUGE M, POULSEN H, HALBERG A, MIKKELSEN M: The value of fluorescence markers in the distinction between maternal and fetal chromosomes. *Humangenetik* 26:187–191, 1975
32. NIIKAWA N, KAJII T: Sequential Q- and acridine orange-marker technique. *Humangenetik* 30:83–90, 1975
33. VERMA RS, LUBS HA: A simple R banding technique. *Am J Hum Genet* 27:110–117, 1975
34. VERMA RS, LUBS HA: Variation in human acrocentric chromosomes with acridine orange reverse banding. *Humangenetik* 30:225–235, 1975
35. MADAN K, BOBROW M: Structural variation in chromosome no. 9. *Ann Genet* 17:81–86, 1974
36. SOKAL RR, ROHLF FJ: *Biometry*. San Francisco, Freeman, 1969, p 156
37. PALMER CG, RIVAS M, WANG L, STINE R: Chromosome polymorphisms in chromosomes 1, 9, and 16. *Mammal Chrom Newsl* 16:93, 1975
38. PARK J, ANTLEY RM: C-band chromosomal polymorphism in Orientals (abstr.). *Am J Hum Genet* 26:65A, 1974
39. HOLZER S, ROSENKRANZ W, GLATZL J: Homozygous duplication on long arm of chromosome pair no. 1. *Humangenetik* 16:341–343, 1972
40. PARK J: Human C-band chromosomal polymorphisms: frequency and distribution in Orientals. Ph.D. thesis, Indianapolis, Indiana University, 1976, p 155
41. WAHLSTROM J. Human chromosomes and fluorescence. *Humangenetik* 12:77–78, 1971
42. SCHNEDL W: Unterschiedliche Fluorescencz der beiden homologen Chromosomen Nr. 3 beim Menschen. *Humangenetik* 12:59–63, 1971
43. ALLEN G: The M quadruplets: probability of uniovular origin judged from qualitative traits. *Acta Genet Med Gemellol (Roma)* 9:240–254, 1960
44. BULMER MG: *The Biology of Twinning in Man,* Oxford, Clarendon Press, 1970
45. GARONER IC, NEWMAN HH: Studies of quadruplets. V. The Kaspar quadruplets. *Heredity* 34:27–32, 1943