Am J Hum Genet 30:85–89, 1978

Polymorphism of Chromosome 9 in 600 Greek Subjects

CATHERINE METAXOTOU,[1] ARIADNI KALPINI-MAVROU, MARIA PANAGOU, AND CHRISTINA TSENGHI

INTRODUCTION

Polymorphisms in human chromosomes are associated with constitutive heterochromatin which is near the centromere of every chromosome, the short arms, and satellite regions of the acrocentric chromosomes, and the distal part of the long arm of the Y chromosome. C-Band heterochromatin is thought to contain repetitive DNA sequences, and the observed variation is primarily due to the sequence length, the number of repeated sequences, and the base composition of the repetitive DNA [1]. Chromosome 9 shows a high frequency of structural rearrangements, such as pericentric inversions and elongated secondary constrictions, which are transmitted as dominant characteristics [2, 3]. The incidence of polymorphic variants for the heterochromatic region of chromosome 9 has demonstrated great variability (in the length and location of the secondary constriction) in newborn populations [4–6] and in patients referred for cytogenetic investigation [2, 3, 7, 8].

This paper presents the frequency of C-band heterochromatin variants of chromosome 9 in 600 nonrandomly selected Greek subjects referred to the cytogenetic laboratory of the 1st Department of Pediatrics, Athens University.

MATERIALS AND METHODS

Table 1 lists the individuals in the present study by reason of referral. Chromosome preparations were made from peripheral blood lymphocyte cultures using conventional techniques.

C-Banding, using the G-11 staining technique [9], was done after G or Q staining on replicate slides. Ten well-spread mitoses from each patient were photographed; the negatives were projected on a screen, and C-band variants were scored visually on the basis of size and location by one individual. Since there was little cell-to-cell variability, a variant was scored as positive if it was present in at least seven of the 10 cells projected. C-Band variants were also checked from at least three photographic prints. As it is difficult to compare chromosomes from different mitotic figures, the long arms of chromosome 21 were arbitrarily used as a genetic landmark for comparing the length of the heterochromatic region [4]. The 10 samples of chromosome 9 are considered as a unit hereafter.

Received November 22, 1976; revised May 2, 1977.

[1] All authors: 1st Department of Pediatrics, Athens University, Aghia Sophia Children's Hospital, Athens 608, Greece.

TABLE 1

CLASSIFICATION OF SUBJECTS IN THE PRESENT STUDY BY REASON OF REFERRAL

Condition	Tested	Pericentric Inversion	9qh 2×21q
Congenital malformations and mental retardation	136	5	3
Sex problems*	118	5	3
Individuals with multiple abortions and stillbirths	127	7	4
Parents of children with Down syndrome and other congenital malformations	107	6	2
Miscellaneous	67	1	. . .
Normal individuals	38	1	. . .
Relatives of probands with inversion or 9qh+	7	(2)†	(1)
Total	600	25	12

* Primary amenorrhea, Klinefelter syndrome, and abnormal sex differentiation.
† Nos. in parentheses are not included in the totals.

RESULTS

Analysis of the data revealed great variation in the length and location of C-bands of the no. 9 chromosomes (table 2). Using the categories and classifications in table 2, we compared the chromosome 9 homologues in 600 individuals. The results of these comparisons can be seen in table 3. No great variation was found in the length of the heterochromatic region of the homologous pairs (table 4). Total inversions and greatly elongated secondary constrictions were only detected in heterozygous individuals.

DISCUSSION

It is generally accepted today [1, 5, 10, 11] that morphologic variations of the C-band region, which are polymorphic since they occur at a high frequency in humans, are inherited and may vary in different populations [2, 6, 12]. Our data revealed extensive variation in the location and length of the heterochromatic region of chromosome 9 (fig. 1).

The reported incidence of pericentric inversions of chromosome 9 has varied

TABLE 2

C-BAND CHARACTERISTICS OF NO. 9 CHROMOSOMES

C-Band Location	No.*(%)	C-Band Length Compared to Length of 21 q†	No.(%)*
No inversion	976 (81.4)	One-fourth 21q	95 (7.9)
Small inversion‡	173 (14.4)	One-half 21q	452 (37.7)
Partial inversion‡	26 (2.2)	Equal to 21q	498 (41.5)
Complete inversion	25 (2.0)	1½ × 21q	143 (11.9)
		2 × 21q	12 (1.0)
Total	1,200 (100.0)	Total	1,200 (100.0)

* Each no. 9 chromosome was examined as a unit.
† The size of 21q was arbitrarily selected for comparison.
‡ Small and partial inversions are considered as variables in the location of the C-band.

TABLE 3

COMPARISON OF C-BAND LOCATION OF NO. 9 HOMOLOGUES IN 600 INDIVIDUALS

IDENTICAL LOCATION OF HOMOLOGOUS PAIR	No.	UNEQUAL LOCATION OF HOMOLOGOUS PAIR		No.
		First Homolog	Second Homolog	
No inversion	389	Small inversion	No inversion	149
Small inversion	12	Partial inversion	No inversion	25
	—	Partial inversion	Complete inversion ..	1
Total	401	Complete inversion	No inversion	24
		Total		199

depending on the population studied and the staining techniques used. De la Chapelle et al. [2] reported a 1% incidence in the Finnish population, and Madan and Bobrow [12] reported 1% in the British population. Lubs and Ruddle [6] reported a more than 20-fold higher incidence in American Negroes than American Caucasians. Recently, McKenzie and Lubs [5] suggested that 4% is probably a minimal estimate, while Hansmann [3] suggested a 5% incidence of human population. Our finding of 25 total pericentric inversions in 600 Greek individuals (4%) is in close agreement with those reported in the literature.

In addition, 25 of the examined individuals (4%) carried a partial inversion with approximately one-third of the constriction located on the short arms (fig. 1). Modified inversions of chromosome 9 have also been observed [1, 3, 4] and may have been produced by breakage within the constitutive heterochromatin. Müller et al. [4] noted a higher incidence of partial inversions in their group (10.7% and 2.2%, respectively for individual chromosomes). In 14.4% of the chromosomes 9 examined, a small part of the heterochromatic region was located on the short arms. Müller et al. [4] also report a higher frequency in their sample (46.2%).

The incidence of elongated heterochromatic regions in the group examined was 7.5%. Others [7, 13] indicated that the increase in the heterochromatic region of chromosome 9 in children with congenital malformations or in relatives of probands with chromosomal anomalies is significantly higher than in normal subjects.

Reports similar to ours are based on investigation of normal subjects [4–6, 11] and patients referred for cytogenetic investigation [2, 3, 7, 8], and no significant differences have been found in the incidence reported for chromosome 9 variants.

TABLE 4

COMPARISON OF LOCATION AND LENGTH OF C-BAND IN HOMOLOGOUS PAIRS OF NO. 9 CHROMOSOMES

	LOCATION	LENGTH	LOCATION AND LENGTH
HOMOLOGOUS PAIRS	No. (%)	No. (%)	No. (%)
Equal	401 (66.7)	251 (41.9)	177 (29.5)
Unequal	199 (33.3)	349 (58.1)	423 (70.5)

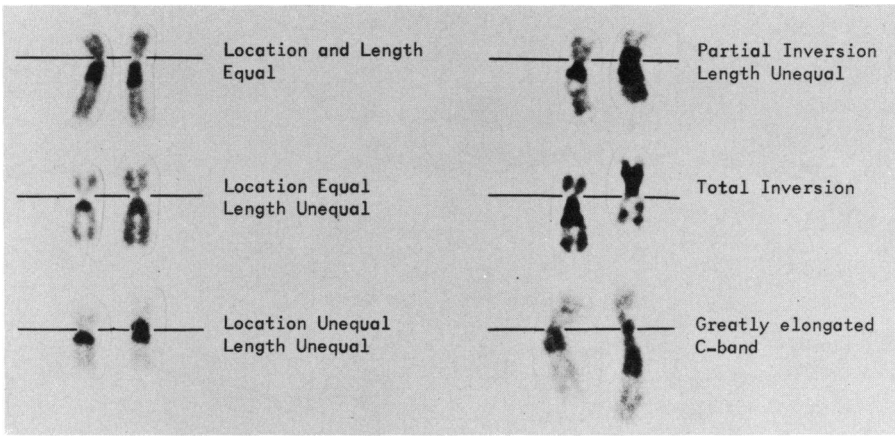

FIG. 1.— Variation in the location and length of the heterochromatic region of chromosome 9.

Nevertheless, we are in the process of examining members of the general Greek population in order to establish that our figures are not influenced by selection.

SUMMARY

The morphologic variations of C-band heterochromatin of chromosome 9 were studied in 600 Greek subjects referred for cytogenetic investigation. There was great variability in the location and length of the heterochromatic bands. The location and length of the heterochromatic band were equal in 177 individuals and unequal in 423. Twenty-five subjects (4%) carried a pericentric inversion on one of the homologs, and 12 individuals (2%) had an extremely elongated C-band in one homolog 9. Our findings are compared with those reported in the literature, and the ethnic variation is discussed and evaluated.

REFERENCES

1. 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
2. DE LA CHAPELLE A, SCHRÖDER J, STENSTRAND K, FELLMAN J, HERVA R, SAARNI M, ANTTOLAINEN I, TALLILA I, TERVILÄ L, HUSA L, TALLQVIST G, ROBSON EB, COOK PJL, SANGER R: Pericentric inversions of human chromosomes 9 and 10. *Am J Hum Genet* 26:746–766, 1974
3. HANSMANN I: Structural variability of human chromosome 9 in relation to its evolution. *Hum Genet* 31:247–262, 1976
4. MÜLLER HJ, KLINGER HP, GLASSER RM: Chromosome polymorphism in a human newborn population. II. Potentials of polymorphic chromosome variants for characterizing the idiogram of an individual. *Cytogenet Cell Genet* 15(4):239–255, 1975
5. MCKENZIE WH, LUBS HA: Human Q and C chromosomal variations: distribution and incidence. *Cytogenet Cell Genet* 14(2):97–115, 1975
6. LUBS HA, RUDDLE FH: Chromosome polymorphism in American Negro and white populations. *Nature* 233:134–136, 1971

7. 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
8. BOUÉ J, TAILLEMITE JL, HAZAEL-MASSIEUX P, LEONARD C, BOUÉ A: Association of pericentric inversion of chromosome 9 and reproductive failure in ten unrelated families. *Humangenetik* 30:217–224, 1975
9. BOBROW M, MADAN K, PEARSON PL: Staining of some specific regions of human chromosomes, particularly the secondary constriction of no. 9. *Nature* [New Biol] 238:122, 1972
10. 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
11. GHOSH PK, SINGH IP: Morphologic variability of human chromosomes: polymorphism of constitutive heterochromatin. *Hum Genet* 32:149–154, 1976
12. MADAN K, BOBROW M: Structural variation in chromosome 9. *Ann Genet* 17:81–86, 1974
13. KUNZE J, MAU G: A_1 and C_9 marker chromosomes in children with combined minor and major malformations. *Lancet* 1:273, 1975

Symposium on the Prevention of Genetic Diseases and Developmental Disabilities

A symposium on the "Prevention of Genetic Diseases and Developmental Disabilities will be held at the Annual Meeting of the American Association for the Advancement of Sciences, February 13, 1978, at the Shoreham Americana Hotel, Washington, D.C. For information contact T. L. Sadick, Ph.D, and S. M. Pueschel, M.D., Child Development Center, Rhode Island Hospital, Providence, Rhode Island.