exception of Alaska, Washington, and Wyoming from which no replies were received. Of the 738 replies that were received, 475 knew the Rh-ABO blood type of the mother, 419 knew the Rh-ABO blood type of the father, and 375 knew the Rh-ABO blood type of both the mother and father.

When compared to the expected frequency of occurrence of Rh blood type in a random sample of the population as reported by Wiener and Socha, there is a significant increase in the incidence of Rh− blood type in the population of mothers of children with spina bifida ($\chi^2 = 117.59$ $p < 0.001$). There were approximately 2.15 times as many mothers with Rh− blood type in this population than would be expected with a random sample of the general population. There was no significant difference in the birth order of the child with spina bifida, when comparing the mothers with Rh− and Rh+ phenotype. There were no reports of perinatal jaundice and/or prenatal or perinatal transfusions. The frequency of occurrence of the fathers' Rh and ABO blood types and the mothers' ABO blood types did not differ significantly from that expected in a random sample of the general population. There was also not a significant increase in spina bifida children in ABO incompatible pregnancies (i.e. mother A, father B or 0; mother B, father A or 0; mother AB, father A, B, or 0).

Discussion. It seems clear that, at least for the population sampled by the questionnaire, there is a significant relationship between maternal RH phenotype and the birth of a baby with spina bifida. Unfortunately, we were not able to obtain any information about anti-Rh antibodies in the mothers with Rh-blood type. However, since birth order of the child with spina bifida is not a significant factor and since there are no reports of perinatal jaundice and/or prenatal or postnatal transfusions, it seems unlikely that this relationship is based on an immunological response to an Rh blood type incompatibility between the mother and father. Clearly, since Rh genotypes are complex, it would be important to determine if there are one or more genotypes that are prevalent in this group of mothers. We are currently attempting to determine the genotype of a selected sample of mothers in our experimental population.

Frequency of occurrence of Rh and ABO blood types amoung parents of children with spina bifida

	Mother Observed	Theoretical	Father Observed	Theoretical
A+	131	122	28	21
B+	43	43	6	8
AB+	18	17	3	3
0+	138	148	21	26
A−	51	53	124	117
B−	17	19	37	41
AB−	7	7	24	16
0−	70	65	132	143
Rh+*	343**	430	354	356
Rh−*	163**	76	65	63

* Based on Rh blood type without regard for ABO blood type.
** Significant difference between observed and expected values, $p < 0.01$.

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The quantitative analysis of constitutive heterochromatic regions of human chromosomes 1, 9, and 16 in relation to size and inversion heteromorphisms in East Indians

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Summary. 100 normal East Indians were studied by C-banding technique to estimate the frequency of size and inversion heteromorphisms of the secondary constriction regions (h) of human chromosomes 1, 9, and 16, and the data were compared to those of Caucasians and Black Americans.

The heteromorphic nature of the constitutive heterochromatin of the secondary constriction regions (h) of human chromosomes 1, 9 and 16 has been recognized since the early 1960s[1], but the extent of the variation has been explored only recently using various banding techniques[2]. CBG (C-bands by barium hydroxide using Giemsa) technique[3] is most commonly used to examine such variation[4], because the h region stains very darkly and contains primarily repetitive DNA[5,6]. There are also some other selective techniques which are being used to examine the extent of such variation[7-9]. The h regions are heteromorphic in size as well as in position (inversion heteromorphisms)[10]. A large number of family studies indicate that these heteromorphisms are inherited in a Mendelian fashion; nevertheless several studies have implied that apparent non-inherited variation between parent and offspring, somatic mosaicism[11,12] and preferential segregation occur[13,14]. Further, an association of increased size of h

regions with inversion is suggested[15,16]. We decided to study the extent of the variation of h regions in chromosomes 1, 9, and 16 in 100 normal East Indians since it is the 2nd most populous race in the world and further to compare it with a normal population of Caucasians and American Blacks. Size and inversion heteromorphisms have been subdivided into 5 classes using subjectively defined criteria. To our knowledge this is the 1st reported study using the C-banding technique on the chromosomes of East Indians.

The 100 normal East Indians studied were all healthy, unrelated und between the ages of 25 and 45 years. They are immigrants from India and have settled in the United States of America. The type of chromosome heteromorphisms were not known at the time of selection for the study. Chromosome preparations were made from peripheral blood as usual. QFQ (Q-bands by fluorescence using quinacrine) and CBG techniques were performed on each individual as described elsewhere[17–19]. C-bands were obtained with a technique described by Sumner[20] with a few modifications[21]. There are several technical variables associated with the quality of C-bands[22]. All variables were taken into consideration to ensure optimal results. QFQ and CBG cells were photographed on Tri-X pan and on high contrast copy film (Kodak) using a Zeiss photomicroscope II. Prior to C-banding, Q-banding was done for identification purposes since C-banding does not identify the individual chromosomes. At least 25 cells were photographed from each individual.

5 partial karyotypes of chromosome pairs 1, 9 and 16 were made from each individual. Cells were selected on the basis of overall quality, lack of overlaps and clarity of C-bands. Emphasis was given to the 3 best cells of each individual.

It is known that variation in size and position of C-bands represents a continuous distribution, and any division into discrete units is arbitrary. Recently, we suggested a method of classifying these heteromorphisms[15–26]. Size heteromorphisms were classified into 1 of 5 sizes using the short arm of chromosome 16 (i.e., 16p) as a reference standard.

Level 1:	$0.5 \times 16p$	Very small
Level 2:	$0.5-1 \times 16p$	Small
Level 3:	$1-1.5 \times 16p$	Intermediate
Level 4:	$1.5-2 \times 16p$	Large
Level 5:	$2 \times 16p$	Very large.

It has proven necessary to have within-cell standards; otherwise, if homologues differ, it is difficult to determine whether one is small or the other large or whether both are variants. The 5 sizes are: (1) very small; (2) small; (3) intermediate; (4) large and (5) very large.

Furthermore, we have proposed a classification of inversion heteromorphisms into 5 categories[15]: (1) h region confined to the long arm-no inversion (NI); (2) less than half the h region present on the short arm-minor partial inversion (MIN); (3) half the h region present on the short arm and the other half on the long arm-half inversion (HI); (4) more than half of the h region present on the short arm-major partial inversion (MAJ); and (5) complete shift of the h region from the long arm to the short arm-complete inversion (CI). The pictorial demonstration of different sizes and the inversion heteromorphisms have been demonstrated earlier[15,26].

Partial karyotypes were scored with respect to size and position of C-band regions on different occasions, and readings were compared with each other. If different readings were noted, more cells were examined from that individual to minimize the technical bias. To study racial differences, this population was compared with data of normal Caucasian[15] and American Black populations[16].

The sizes of the h regions of chromosomes 1, 9, and 16 are classified into 5 categories in the table. A method for calculating the frequencies of the heteromorphisms has been described elsewhere[23]. Any class which contributed less than 25% of the total is considered as a heteromorphic class. The frequencies of the size heteromorphisms of the h regions of chromosomes 1, 9, and 16 are 16.0%, 32.0% and 6.5% respectively in East Indians. Data are also compared with those for Caucasians and Blacks in the table. For chromosome 1, a higher incidence ($p < 0.01$) of heteromorphisms was noted in Indians (16.0%) while Caucasians and Blacks exhibited similar but lower frequencies (11.25% and 10.36%). For chromosome 9 a significantly higher ($p < 0.01$) incidence was noted in Caucasians while Indians and Blacks had a similar incidence ($p < 0.01$). Chromosome 16 was the least heteromorphic in all groups and no racial variation was noted.

Data on C-band heteromorphisms in East Indians are not available, therefore, comparison with race cannot be provided. Lubs et al.[24] classified constitutive heterochromatin regions into 3 size categories normal (N), large (+) and small (−) using 190 Blacks and 194 Caucasians with different IQs. On this basis they concluded that there was no racial differences in either extremely small or large h regions of chromosomes 1, 9 and 16. However, when chromosomes are classified in only 3 categories, many groups would be placed in the same class although they differed in C-band size. More recently, a population of 403 mentally retarded individuals from diverse ethnic groups (except Blacks and Indians) was studied by Matsuura et al.[25]. The authors concluded that Orientals have a larger C-band on chromosome 9 than the other racial groups, while

Comparison of percent hetermorphisms among east Indians (I), American Blacks (B) and Caucasians (C)

Size	1			9			16		
	I	C	B	I	C	B	I	C	B
Very small (1)	14	10.00	10.00	7.0	24.37	13.75	37.0	48.12	40.0
Small (2)	57	43.75	59.37	68.0	52.50	70.00	56.5	44.38	53.12
Intermediate (3)	27	45.00	30.00	23.5	21.25	14.37	6.0	7.5	5.00
Large (4)	2	1.25	0.36	1.5	1.88	1.88	0.5	0.0	1.25
Very large (5)	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.63
% Heteromorphisms	16.0	11.25	10.36	32.0	47.5	30.0	6.5	7.5	6.80
Inversion									
NI (1)	82.5	90.0	82.5	79.0	88.75	78.1	98.5	0	0
MIN (2)	13.5	9.4	14.4	12.0	8.75	13.1	1.0	0	0
HI (3)	3.0	0.6	3.1	8.5	2.50	7.5	0.5	0	0
MAJ (4)	0.0	0.0	0.0	0.5	0.0	0.0	0.0	0	0
CI (5)	1.0	0.0	0.0	0.0	0.0	1.3	0.0	0	0
% Heteromorphisms	17.5	10.0	17.5	21.0	11.25	21.9	1.5	0	0

Caucasians have a large C-band on chromosome 9. The sum of the C-bands on chromosomes 1, 9 and 16 in different racial groups were 16.57% (Oriental), 17.07% (Caucasian), 16.30% (Filipino), 15.84% (Polynesian), 16.30% (other)[25].

Further, we report the location of h regions since they have a tendency for pericentric inversion. We have proposed a classification of such heteromorphisms into 5 categories. The frequencies of the 5 categories found in 100 East Indians are recorded in the table and data are also compared with Caucasians and Blacks. For chromosomes 1 and 9, East Indians and Blacks have similar frequencies of inversion heteromorphisms while Caucasians have a significantly ($p < 0.01$) lower incidence of this type of heteromorphisms. Inversion of the h region in chromosome 16 is very rare and was not found in Black and Caucasian populations while 1.5% of chromosome 16 in the East Indian population showed inversion.

The biological and clinical implications of such heteromorphisms are poorly understood. There are preliminary indications that certain of the rare heteromorphisms may carry an increased risk, like mental retardation, fetal wastage, infertility, etc.[2]. Racial differences may be of anthropological interest and are of great value in linkage and population studies. Other important applications of heteromorphisms are to study the mechanism of mosaicism, paternity testing, maternal cell contamination during amniocentesis, and identifying the transmission from one of the parents of a specific chromosome that may be carrying a deleterious gene. The present study provides base line data in a normal population for comparing size and inversion heteromorphisms with an abnormal population.

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The effect of L-cysteine on presoaked barley seeds treated with methyl methanesulfonate

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Summary. Experiments were performed to analyse the effect of L-cysteine on barley seeds treated with methyl methanesulfonate (MMS) at a critical stage of the cellular cycle. Contrary to expectation, L-cysteine did not protect the barley seeds against MMS damage except for a slight protection at high doses (0.5, 0.7%).

It has been reported that applying mutagenic treatments at the beginning of DNA synthesis (S) in barley seeds increases mutation frequency and reduces physiological damage[2–4]. Cysteine has been used with success as a protector against the toxicity of alkylating agents in higher plants[5–8], and it is known to be capable of acting at all stages of the cellular cycle[9]. Methyl methanesulfonate, together with L-cysteine, was previously shown to exhibit a synergistic effect on lethality rather than conferring protection in bacteria[10]. This experiment was designed to determine the action of L-cysteine at a critical stage of barley seed germination (end of G_1 and onset of S) while subjected to methyl methanesulfonate (MMS) treatment.

Batches of 130–210 barley seeds (variety 'comun') were presoaked for 15 h in demineralized water at 20 °C in order to reach the end of G_1 and the onset of S[4,11]. The seeds were then treated for 1 h at 20 °C in 0.2, 0.5 and 0.7% w/v aqueous solutions of MMS (Merck) and then washed for 5 min in tap water. Each seed lot treated with MMS was then post-treated during 1 h in a 0.01 M aqueous L-cysteine (Merck) solution also at 20 °C. Both MMS and L-cysteine solutions were prepared with demineralized water and used fresh. Since L-cysteine is easily oxidized to cystine, the L-cysteine solution was prepared with boiled water in order to remove dissolved oxygen. To test for oxidation to cystine, TLC was performed according to the method of Slaten et al.[12] at 0, 2, 4, and 18 h. No oxidation to cystine was detected at 0 and 2 h, but at 4 and 18 h a progressive transformation of L-cysteine to cystine was noted. Because our post-treatment period was only 1 h long, no precautions were deemed necessary.

For homogeneity, the MMS treatments were carried out in