Racial variation of a non-fluorescent segment of the Y chromosome in East Indians*

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SUMMARY Seventy normal male East Indians were selected to study the length of the fluorescent (f), non-fluorescent (nf), and total length of the Y chromosome by the QFQ technique. The length of the Y chromosome was classified into five groups using subjectively defined criteria, namely, very small, small, average, large, and very large. The frequencies of Y/F indices for these groups were 0.0%, 1.42%, 15.71%, 58.57%, and 24.28%, respectively. The variation in the total length of the Y chromosome was accounted for by variations in the length of the nf as well as the f segments. The mean Y/F, f/F, and nf/F indices for East Indians were 1.20, 0.47, and 0.73, respectively. A longer Y was noted in East Indians primarily owing to an increase in size of the nf segment in comparison to normal black and Caucasian populations. Furthermore, it is emphasised that the definition of 'average' or 'large' Y varies from race to race.

Size variation (polymorphism or heteromorphism) of the long arm of the human Y chromosome from person to person and even from one ethnic group to another was established even before the banding era,¹⁻³ and its inheritance at constant length is a documented fact.⁴⁻⁶ By the QFQ (Q bands by fluorescence using quinacrine) technique,^{7,8} the long arm of the human Y (Yq) chromosome differentiates into two segments, namely non-fluorescent (nf) and fluorescent (f), and the two segments have been designated Yq11 and Yq12, respectively.⁹ The variation in the length of the Y chromosome has previously been reported as resulting from the f segment while the nf segment remained constant.¹⁰⁻¹⁴ However, three preliminary studies, including one of our own, indicate that the nf segment may also be responsible for the length variation.¹⁵⁻¹⁷ Our preliminary survey of the chromosomes of East Indians suggested the presence of a longer Y chromosome. This provided us with a unique opportunity to determine whether the nf segment as well as the f segment is variable in size and, if so, whether the increased size of the Y chromosome is determined by an increase in one or both segments. Furthermore, the degree of size

correlation (r) between the f and nf segments was measured using extensive statistical analyses.

Materials and methods

The 70 East Indians were all healthy and between the ages of 28 and 45 years. Sixty Caucasian and 60 American blacks were taken from our previous survey.^{17,18} All subjects had negative medical histories and were unrelated. The length of the Y chromosome was not known at the time of selection for the study. All chromosome preparations were made from cultured peripheral blood.¹⁹ The QFQ technique was carried out 3 to 7 days after harvesting. QFQ cells were photographed on tri-X pan film using a Zeiss photomicroscope II.^{20,21} At least 20 to 30 cells were photographed from each person and more than 4500 cells were photographed from the three populations.

The five best differentiated cells were selected from each person. Chromosomes were measured directly from the negative film, as a considerable amount of information is lost during printing. All possible variables were taken into consideration to ensure optimal results.^{22,23} Cells were projected by a Simmon Omega Point Light Source Enlarger (Simmon Omega Inc, NY, magnification $\times 8000$). All chromosomes 19 and 20 (that is, F group chromosomes) were measured. The total Y length and the fluorescent (f) and non-fluorescent (nf)

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segments were measured in the same cell. The value of F was based on the average lengths of chromosomes 19 and 20. From these measurements, the ratio Y/F, f/F, and nf/F were determined for each cell and the average was taken from five cells.

In order to determine the 'functional relationship' of one variable (for example, length of f segment vs total length of Y, etc) with another, a regression coefficient analysis was performed. A 'function' is a mathematical relation enabling us to predict what value of a variable Y corresponds to given values of a variable f, etc. A Y intercept (a) and regression coefficient (b) was calculated to determine the regression equation. A test of significance of the regression coefficient was also performed. Correlation coefficient (r) analysis was used to examine the degree to which two variables vary together. Once established, such an association is likely to lead to reasoning about causal relations between the variables. Test of significance and confidence limits for correlations (r) were also calculated. Since we are dealing with proportions, the 'angular transformation' was used to test the significance of nf as well as f segments of the Y chromosomes. The frequency distribution was also tested by the χ^2 test. Confidence limits and standard deviations of means were also calculated to provide better magnitudes of statistical parameters.²⁴

Results and discussion

The length of the human Y chromosome was classified into five classes, namely very small, small, average, large, and very large. The logic behind this classification has been suggested elsewhere.^{17,18} The previous surveys were done by using similar conditions and criteria to describe the variation of the human Y chromosome. The frequency distribution of the Y/F index within each class interval is compared in table 1 with two other groups. There was only 1.42% of East Indians that had a small Y, while this class was 15% in Caucasians and 3.33% in blacks. The distribution of Y/F

FIG 1 Distribution of Y/F indices among East Indians (▲), Caucasians (●), and American blacks (■). The data are taken from table 2.

indices of the five classes is shown in fig 1 and it is clearly demonstrated that there was a normal distribution of Y/F indices among Caucasians and Indians, while the black population showed a skewed curve (skewed to the left). Therefore, to compare the average length of the Y chromosome from one group to another is inappropriate. At this point it would be useful to determine the significance of the categories. Obviously, the definition of a large Y will depend upon what race one is dealing with. For example, if we say that average should represent the most frequent class (with Y/F index of 0.95 to 1.09), then only Caucasians and blacks will conform to this. In the Indian population, the most frequent class was large, while average was only third in frequency.

Long Y chromosomes, according to the definition of Court-Brown,²⁵ are either as long as or longer than F group (19 and 20) chromosomes. Generally, the length of the Y chromosome has been classified into two categories, short or long, depending on the value of the Y/F index but, in fact, a continuous variation exists which might be subdivided in many ways. As we have clearly demonstrated, racial differences can be demonstrated using five classes. Using five categories, we further demonstrated that the definition of the size of the Y as small or large will depend upon race. For example, if an East

TABLE 1 Classification of Y/F indices from normal Caucasians, American blacks, and East Indians.

Criteria (Y/F indices)	Size description	Size distribution					
		Sample size			Frequency (%)		
		C (n = 60)	B (n = 60)	I (n = 70)	C	B	I
≤ 0.80	Very small	0	0	0	0	0	0
0.81-0.94	Small	9	2	1	15.0	3.33	1.42
0.95-1.09	Average	40	34	11	66.7	56.67	15.71
1.10-1.23	Large	8	18	41	13.3	30.00	58.57
> 1.23	Very large	3	6	17	5.0	10.00	24.28

C = Caucasian, B = American black, I = East Indian.

FIG 2 The length of the fluorescent segment of the Y chromosome (f/F) plotted against the length of the Y chromosome (Y/F). Regression line is also drawn.

FIG 3 The length of the non-fluorescent segment of the Y chromosome (nf/F) plotted against the length of the Y chromosome (Y/F). Regression line is also drawn.

Indian has a Y which falls in the class of 1.10 to 1.23, his Y will be called average, while that of a Caucasian or black will be called large.

In an attempt to determine the reason for the longer Y in East Indians, the size of the f and nf segments were considered. To examine the heteromorphisms of f and nf segments of the Y chromosome, f/F and nf/F ratios were also calculated. If the nf segment of the Y chromosome is constant in size, then the nf/F ratio should not change, since the length of the F group chromosomes is constant.^{9,26} A functional relation between the two variables (f/F vs Y/F) was calculated by using regression coefficient analysis and a regression line was established (fig 2). A test of significance of the regression coefficient was also performed, and it was concluded that highly significant positive regression was present ($p < 0.01$). The correlation coefficient (r) was also calculated and tested by the t test. The r was 0.93 ($p < 0.01$) and the 95% confidence limit was computed to be 0.8937 to 0.9571 for f/F vs Y/F . Consequently, it was concluded that there was a significant correlation between the fluorescent segment and the total length of the Y chromosome. Similar statistics were computed for nf vs Y/F and a similar conclusion was reached. The correlation coefficient was 0.64 ($p < 0.01$) with a confidence limit of 0.4754 to 0.7595. The graphic plot is shown in fig 3. The correlation coefficient was also calculated for f/F vs nf/F indices ($r = 0.05$) and no significant relation was observed ($p > 0.05$), that is, the f and nf segments varied independently and there was no relation between them. A graphic plot is shown in fig 4. The mean Y/F , f/F , and nf/F indices are 1.20, 0.47, and 0.73, respectively (table 2).

Our findings indicate that the length of the Y chromosome is dependent on the nf as well as the f segments. Thus, both segments account for an

FIG 4 The length of the non-fluorescent segment of the Y chromosome (nf/F) plotted against the length of the fluorescent segment (f/F). No relationship was observed between these two parameters (see text).

increase in the size of the Y. However, the longer Y in East Indians was primarily due to the non-fluorescent segment. There was a significant difference in the length of the nf segment in Indians when compared with Caucasians ($p < 0.05$). Comparison with blacks will not be appropriate since their values are not normally distributed. Furthermore, using the $2 \times N$ contingency χ^2 test, the distribution of Y lengths are significantly different ($p < 0.05$). These findings are contrary to the earlier contention that the fluorescent segment accounts for the size variation of the Y chromosome while the non-fluorescent region is stable.¹⁰ No relation between the size of the f and nf segments was observed; in other words they varied independently.

In man only two genes have been assigned to the Y chromosome.^{27,28} The TDF (testis determining factor) is located on the short arm while the H-Y (histocompatibility antigen) gene is located in the non-fluorescent (nf) segment of the long arm of the

TABLE 2 Comparison of statistical parameters between Caucasians, American blacks, and East Indians.

Components	Caucasians	American blacks	East Indians
Index			
Y/F	1.02±0.09	1.09±0.10	1.20±0.10
f/F	0.44±0.07	0.42±0.09	0.47±0.08
nf/F	0.57±0.04	0.67±0.04	0.73±0.06
Correlation (r)			
f/F vs Y/F	0.86	0.89	0.93
nf/F vs Y/F	0.63	0.53	0.64
f/F vs nf/F	0.26	0.22	0.05
Confidence limit			
f/F vs Y/F	0.9154-0.7818	0.9329-0.8210	0.9571-0.8937
nf/F vs Y/F	0.7616-0.3876	0.6874-0.3131	0.7595-0.4754
f/F vs nf/F	0.4831-0.0750	0.4470-0.0385	0.2840-0.1868

chromosome. This raises the question of whether the size of the nf segment has any phenotypic effect, especially when it is either too long or too short. The present study suggests that nf and f segments do not have any clinical effect. The reason for the heteromorphic size of the long arm of the Y chromosome is generally thought to be the result of deletion.²⁹⁻³² Muldal and Ockey³⁰ came to this conclusion because of the lack of the distal portion of the Y chromosome. Wahlstrom³³ observed that extremely short Y chromosomes did not fluoresce brightly at their distal end by the QFQ technique. There is sufficient evidence to conclude that the bright segment (f) contains large amounts of repetitive DNA³⁴⁻³⁶ and can be further subdivided into several bands^{37,38}; the size variation in these sub-bands could be a repeat in the tandem arrangement. The mechanism generating length variation of the nf segment is not clear at present.

The biological significance of variation in the length of the Y chromosome is not clear.³⁹ The observed group differences might indeed represent true chromosomal heteromorphisms, but the factor that maintains this heteromorphism in the population is not known.⁴⁰ Lubs and Patil⁴¹ suggested that there exists a north/south gradient in the length of the Y in Europeans, men of Mediterranean origin having a longer Y. Furthermore, some investigators have found a longer Y in criminals,⁴² while others have not found any length differences between criminal and non-criminal controls.^{43,44} It has also been suggested that long Y chromosomes may be an important cause of fetal loss.^{45,46} Recently, a higher incidence of a small Y chromosome in humans with trisomy 21 has been reported and it is speculated that fathers with a small Y chromosome might be at higher risk for non-disjunction for chromosome 21.⁴⁷

The present study clearly demonstrates that racial variation in the length of the Y chromosome is the result of variation in both the non-fluorescent and

fluorescent segments, and future studies should attempt to determine if variation in the nf segment plays a role in fetal loss or other clinical parameters. In summary, this study provides measurement data on nf and f segments in a normal East Indian population and compares these data with other normal as well as abnormal populations.

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