Length heteromorphisms of fluorescent (f) and non-fluorescent (nf) segments of human Y chromosome: classification, frequencies, and incidence in normal Caucasians¹

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SUMMARY Sixty normal male Caucasians were selected to study the length of the Y chromosome. QFQ banding was performed. Chromosomes 19 and 20 (F) and Y were measured directly from the film. Y/F, f/F, and nf/F indices (f = fluorescent; nf = non-fluorescent segment) were determined. The length of the Y chromosome was classified into 5 groups: very small, small, average, large, and very large with Y/F indices of <0.8, 0.81-0.94, 0.95-1.09, 1.1-1.23, and >1.23, respectively. The frequencies of Y/F indices for these groups were 0 (0%), 9 (15.0%), 40 (66.7%), 8 (13.3%), and 3 (5.0%), respectively. The most frequent class was 0.95-1.09 and was defined as the 'average' Y/F index for the human Y chromosome. The variation in the total length of the Y chromosome was accounted for by variations in the length of the non-fluorescent as well as the fluorescent segments. No relation between f and nf segments was observed. The mean Y/F, f/F, and nf/F indices were 1.022, 0.441, and 0.574, respectively.

Even before the advent of banding techniques, the length of the human Y chromosome was known to vary from person to person and from one ethnic group to another (Cohen *et al.*, 1966). It has been concluded from family studies that the Y chromosome is inherited at a constant length (Bishop *et al.*, 1962; Unnerus *et al.*, 1967; Borgaonkar *et al.*, 1969; McKenzie *et al.*, 1972). The distal two-thirds of the long arm of the human Y chromosome is brightly fluorescent when the QFQ (Q bands by fluorescence using quinacrine, as suggested by Paris Conference, 1971; Supplement, 1975) banding technique is employed. It has been suggested that the genetically active material is located in the non-fluorescent segment and this segment has been considered invariable in size

(Bobrow *et al.*, 1971; Laberge and Gagne, 1971; Knuutila and Grippenbergh, 1972), while the genetically inactive brilliant fluorescent segment is variable in size (Paris Conference, 1971). There is some evidence, however, that there is a possible variation in the length of the non-fluorescent segment (Schnedl, 1971; Soudek *et al.*, 1973).

The biological and clinical significance of human Y chromosome heteromorphisms is poorly understood. Lubs and Patil (1975) suggested that there exists a north/south gradient in the length of Y in Europeans; men of Mediterranean origin have a longer Y. Furthermore, some investigators have found a longer Y in criminals (Soudek and Laraya, 1974) while others have not found any length difference between criminal and non-criminal controls (Benezech *et al.*, 1976; Brogger *et al.*, 1977). Patil and Lubs (1977) have suggested that long Y chromosomes may be an important cause of fetal loss.

Long Y chromosomes, according to the definition of

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Court Brown (1967), are either as long as or longer than F chromosomes. Generally the length of the Y chromosome has been classified into two categories, that is short or long depending on the value of the Y/F index, but, in fact, a continuous variation probably exists which might be subdivided in many ways. We have studied Y chromosome variation among 60 normal Caucasians. The purpose of this study was:

(i) To define the average length of the human Y chromosome and to classify the different lengths into at least 5 categories.

(ii) To examine the regression and correlation coefficients between fluorescent (f) and non-fluorescent (nf) segments with respect to the Y/F index.

(iii) To determine whether the non-fluorescent as well as the fluorescent 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.

(iv) To measure the degree of size correlation between fluorescent and non-fluorescent segments.

Material and methods

SELECTION OF SUBJECTS

The 60 normal male individuals studied were all healthy Caucasians between the ages of 26 and 65. Forty-five were blood donors from the University of Colorado Medical Center, Denver, Colorado and 15 were blood donors from the Jewish Hospital and Medical Center, Brooklyn, New York. All subjects had negative medical histories and were unrelated.

CYTOGENETIC TECHNIQUES

All chromosome preparations were made from cultured peripheral blood. Culturing and harvesting techniques have been described earlier (Lubs *et al.*, 1973). 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 (Verma and Lubs, 1975, 1976; Verma and Dosik, 1976). At least 20 to 30 cells were photographed from each individual with more than 1500 cells photographed from the total population.

MEASUREMENTS

The 5 best differentiated cells were selected from each individual. Chromosomes were measured directly from the negative as there is a considerable amount of information loss during printing. Cells were projected by a Simmon Omega point light source enlarger (Simmon, Omega, Inc., N.Y., U.S.A., Magnification $\times 8000$). All chromosomes 19 and 20 (F) were measured: the total Y length, the fluorescent (f), and non-fluorescent (nf) 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 ratios Y/F, f/F, and nf/F were determined for each cell and the average was taken from five cells.

STATISTICAL ANALYSIS

In order to determine the 'functional relation' 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 regression coefficient was also performed. We also employed correlation coefficient (r) analysis 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. Tests of significance and confidence limits for correlation coefficients were also calculated (Sokal and Rohlf, 1969).

Results and discussion

It was assumed that the Y/F indices among 60 Caucasians are normally distributed. Based on this hypothesis, the Y/F indices were classified into five class intervals (Table). The most frequent class interval was 0.95 to 1.09 and was referred to as the 'average' length of the Y chromosome. There were 40 (66.7%) subjects who were included in this class interval. The class intervals were defined as very small, small, average, large, and very large. The frequency distribution of Y/F index within the class intervals of very small, small, average, large, and very large was 0 (0%), 9 (15.0%), 40 (66.7%), 8 (13.3%), and 3 (5.0%), respectively (Table). The mean Y/F index was 1.022 ± 0.090 .

To examine the variation (heteromorphisms) of fluorescent (f) and non-fluorescent (nf) segments of the human Y chromosome, f/F and nf/F ratios were 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 chromosome is constant (Paris Conference, 1971; Ledley *et al.*, 1972). A functional relation between the two variables (f/F vs. Y/F) was calculated by using regression

Table Classification of Y/F indices from 60 normal Caucasians

Class intervals (Y/F index)	Frequencies	Sizes
<0.80	0 (0)	Very small
0.81-0.94	9 (15)	Small
0.95-1.09	40 (66.7)	Average
1.10-1.23	8 (13.3)	Large
>1.23	3 (5)	Very large

Fig. 1 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 shown: a = intercept; b = regression coefficient (see Sokal and Rohlf, 1969).

coefficient analysis, and a regression line was established (Fig. 1). 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 was also calculated and tested by the t test. The correlation coefficient was 0.86 and the 95% confidence interval was computed to be 0.782–0.915. It was found that there was significant correlation between f/F and Y/F indices ($P < 0.01$), that is the length of the Y chromosome was significantly correlated with the length of the fluorescent segment. Similar statistics were computed for nf vs. Y/F and a similar conclusion was reached. The correlation coefficient was 0.63 ($P < 0.01$), with confidence interval of 0.388–0.762. The graphic plot is shown in Fig. 2. The correlation coefficient was also calculated for f/F vs. nf/F indices (0.261) and no significant relation was observed ($P > 0.05$), that is f and nf segments vary independently and there was no relation between them. A graphic plot is shown in Fig.

3. The mean f/F and nf/F indices were 0.441 and 0.574, respectively.

Our findings indicate that the length of the Y chromosome is dependent on the nf as well as f segment. Thus both segments account for an increase in the size of the Y. It was suggested earlier that the fluorescent segment accounts for the size variation of the Y chromosome while the non-fluorescent region is stable (Bobrow *et al.*, 1971). No relation between f and nf segments was observed.

In the Paris Conference (1971) the long arm of the Y chromosome was divided into two bands. Recently Jalal *et al.* (1974) and Drets and Seunanez (1974) found that the long arm of the Y chromosome could be divided into 5 bands. It has been generally accepted that dim fluorescent regions contain DNA rich in G-C content while bright fluorescent regions suggest DNA rich in A-T content (Weisblum and DeHaseth, 1972). There is sufficient evidence to conclude that the brightly fluorescent segment contains very high

Fig. 2 The length of the non-fluorescent segment of the Y chromosome (nf/F) plotted against the length of the Y chromosome (Y/F).

Fig. 3 The length of the non-fluorescent segment of the Y chromosome (nf/F) plotted against the length of the fluorescent segment (f/F).

amounts of repetitive DNA (Pardue and Gall, 1970; Arrighi and Hsu, 1971; Yunis *et al.*, 1971). Size variations in this segment could be a repeat in a tandem arrangement. The mechanism generating length variation of the nf segment is at present unclear.

Since our study showed that there is variation in the nf as well as f region of the Y chromosome, future investigation of Y chromosomal abnormalities should attempt to determine if variation in the nf segment plays a role in the abnormalities. Furthermore, these findings make possible a more sophisticated analysis of the clinical significance of long Y chromosomes. Similarly, measurements might help to resolve the conflicting findings; for example, if certain clinical measurements were found to be more closely correlated with increased nf material than with f material or the total Y length. We did not find any structural variation among 60 Caucasians selected for this study.

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