Lymphocyte interphase chromatin in healthy subjects, patients with Down's syndrome, and their parents and sibs

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SUMMARY A study of the interphase chromatin structure of lymphocytes in healthy subjects, patients with Down's syndrome, and their parents and sibs was carried out by AO labelled fluorometry using our modification of DNP cell thermal denaturation. Analysis by the Sperry Univac 90/30-B computer showed that in 40% of healthy subjects the lymphocyte chromatin melting profiles had a regularly repeated curve with six (seven) maxima at definite temperatures. In the remaining 60% some regularly repeated deviations were present and were correlated with the sex of the subject examined. There were five subgroups in the female group and seven subgroups in the male group.

In 97% of patients with Down's syndrome the lymphocyte chromatin melting profiles gave curves with three maxima at temperatures of 65, 85, and 92°C ($\pm 2^\circ$). Maxima at 78 and 45°C were absent.

In 80% of the mothers of probands with Down's syndrome and in 30% of female sibships, lymphocyte melting profiles also produced curves with three maxima: 65, 85, and 92°C ($\pm 2^\circ$). In view of the fact that similar changes were observed in mothers and female sibs only, we propose that some women may have genotypical peculiarities which may possibly contribute to the origin of this chromosome pathology.

The purpose of the investigation described here was to develop a new approach to research into the structure of the interphase chromatin in human cells. By this approach we were able to carry out a comparative analysis of the correlation between karyotype, phenotype, and genotype in healthy subjects, patients with trisomy 21, and their parents and sibs.

Materials and methods

Using a comparative analysis of temperature dependent fluorescence of acridine orange bound to cellular DNP by our modification¹ of the method of Ringertz and Gledhill,² the structure of the interphase chromatin of peripheral blood lymphocytes was examined in the following groups of subjects.

(1) Healthy donors: 164 subjects of whom 98 were males and 66 were females, age range 20 to 45 years.

(2) Fifty patients with Down's syndrome (25 47,XY,+21 and 25 47,XX,+21), age range 8 to 30 years.

(3) Parents of children with Down's syndrome:

50 mothers aged 20 to 48 and 35 fathers aged 20 to 58.

(4) Sibs: 15 girls aged 6 to 22 and 15 boys aged 10 to 24.

Changes of the chromatin structure in response to heating were followed up by the amount of fluorescent label, acridine orange (AO), taken up, with the intervals of reading at 1 to 2°C.

Investigations were carried out using short term cell cultures, that is, cells incubated for one hour in a nutrient medium with 10% autologous serum.

The fluorescence intensity of AO bound to the lymphocyte chromatin DNA was measured by the Opton MSP-0.5 scanning microscope-photometer. Fluorescence was excited with light of wavelength (λ) 365 nm and recorded at $\lambda = 530$ nm by means of interference light filter. Changes in intensity of fluorescence at $\lambda = 640$ nm were recorded simultaneously to define ' α ' ratio = F_{640}/F_{530} . The F_{640} value was negligible. Therefore, only the results of changes for F_{530} are given. The instrument operational details, as well as lymphocyte isolation and

Received for publication 15 March 1982.

cell cultivation, were described by us earlier.¹ The results of fluorescence intensity (F_{530}) of the DNP = AO complex of cells in a temperature range of 20 to 100°C were examined using the Sperry Univac 90-30-B'' computer with a special programme. This was done in order to determine: (1) the statistical significance of the maxima demonstrated at certain temperature intervals; and (2) to distribute into groups all the numerical values of the curves obtained in relation to the coinciding characteristics of the presence or absence of maxima.

Numerical values of the F_{530} DNP = AO complex in 24 points corresponding to the following temperatures: 20, 35, 40, 42, 45, 47, 50, 55, 60, 65, 67, 70, 75, 77, 78, 80, 82, 85, 87, 88, 89, 90, 92, and 95°C were used as initial data for the programme. Each curve was examined with respect to the presence of a maximum in the environs of points corresponding to 45, 65, 78, 88, and 92°C. These environs were: 42, 47, 60, 67, 77, 78, 85, 90, and 92°C. On a fixed curve the characteristics of the presence of a maximum were determined for each of these points: M-maximum, N-absence of maximum, V-uncertainty (absence of data). For this purpose the presence of a maximum was first analysed in each point of the environ separately, the value of a curve being examined in the point analysed and in two adjacent ones. If the value in this point exceeded those in the adjacent ones by more than 2, the presence of a (mild: $\pm 2^\circ$) maximum was noted. In all other cases no maximum was considered to be present. This procedure was applied to all points of the environ. Then all curves were broken up into

classes or groups of curves with coinciding characteristics of the presence of maxima M (or N) in six environs.

Results

HEALTHY SUBJECTS

A computerised analysis of the data obtained (F_{530} DNP-AO complex in the temperature range of 20 to 100°C) showed that, irrespective of sex, in about 40% of cases (± 0.35) the temperature dependent variations in AO fluorescence of interphase chromatin in healthy subjects presented a complicated but regularly repeated picture of a curve with six maxima at the following temperatures: 45, 65 (± 3), 78 (± 1), 85, 88, and 92 (± 2)°C, that is, a clear-cut 'identity' of the above parameter was common to different subjects. The regularity of DNP cell melting profiles was revealed in the most numerous group of healthy subjects, 50% of females and 40% of males (modal class), and was taken by us as the standard normal variant (fig 1).

In the remaining 60% of cases, various but regularly repeated deviations were noted: (1) the absence of a maximum; (2) the merging of two adjacent maxima in one; and (3) the shift of one of the maxima to the left or to the right by more than 3°C.

It should be noted that in most cases the nature of the deviations depended on the sex of the subject examined. In females the most common type of deviation was the merging of two maxima into one:

FIG 1 Lymphocyte interphase chromatin melting profiles of healthy subjects (46%) obtained by acridine orange fluorescence (F_{530}).

78 and 85°C (nine cases), 85 and 88°C (eight cases), and 88 and 92°C (nine cases) (fig 2). As for the coinciding characteristics of the presence of the maxima on the curve of DNP lymphocyte melting, the female control group fell into five subgroups, each containing not less than five identical cases.

Seven subgroups were singled out in the control group of males: the basic group comprised cases with six (seven) maxima revealed at definite temperatures (~40%). The most common type of deviation was the absence of a maximum at 85°C and its appearance at 82°C (25% of the cases) (fig 3). There

were unclassified, strictly individual types of deviations in the female group (5%) and in the male group (17%).

We were thus able, by using a computer, to classify individual cases of interphase chromatin structure by heat denaturation patterns in healthy subjects.

PATIENTS WITH DOWN'S SYNDROME

In response to heat the fluorescence intensity of AO combined with aneuploid genome chromatin increased regularly by 1.5 to 2 times at the following

FIG 2 The most frequent modification of deviations in the lymphocyte interphase chromatin melting profiles of healthy females manifested in the merging of two maxima into one blurred maximum at 78 and 85°C (8%).

FIG 3 The most frequent modification of deviations in the lymphocyte interphase chromatin melting profiles of healthy males manifested in the absence of a maximum at 85°C (25%).

temperatures: 65, 85, and 92°C ($\pm 2^\circ\text{C}$), demonstrating only three maxima in contrast to the six or seven in the controls, regardless of the sex of the subject examined (fig 4).

It should be noted that the major differences in the curves of acridine orange fluorescence against temperature of diploid (healthy donors) and trisomic cells were within the temperature range of 70 to 85°C. Within this temperature interval the patients' cells showed a smooth rise of the curve with a single blurred maximum in the region of 85°C. In 95% of the cases there was no peak at 45°C.

MOTHERS OF PROBANDS WITH DOWN'S SYNDROME AND FEMALE SIBS

In response to heat the lymphocytes of healthy mothers and female sibs (80% and 30% of the cases

respectively) reacted by a regular increase in the AO fluorescence intensity in the temperature ranges of 45, 65, 85, and 92°C ($\pm 2^\circ\text{C}$). Three or four maxima were demonstrable, as was typical of the probands. The maximum at 45°C was either absent or merged with the peak at 65°C (fig 5).

FATHERS OF PROBANDS WITH DOWN'S SYNDROME AND MALE SIBS

No deviations from the standard were demonstrated in the fathers and their healthy sons (fig 6). Thus, certain signs of the changed interphase chromatin structure of patients with Down's syndrome were revealed only in their mothers and healthy female sibs. It is interesting to note that the main indication of the changed chromatin structure in patients with Down's syndrome (double maxima) was also present

FIG 4 Lymphocyte interphase chromatin melting profiles in patients with Down's syndrome (trisomy 21) (97%).

FIG 5 Lymphocyte interphase chromatin melting profiles of the healthy mother of a child with Down's syndrome.

FIG 6 Lymphocyte interphase chromatin melting profiles of fathers (above) and male sibs (below). Abscissa, temperature (in °C). Ordinate, ratio of the fluorescence intensity of AO, combined with human lymphocyte chromatin at a given temperature, to AO fluorescence intensity at 20°C: $F_{530} T^{\circ}C / F_{530} 20^{\circ}C$.

in a proportion (8%) of the healthy female population. The above sign is not correlated with mother's age or with the sequence of birth (before or after the proband) in female sibs.

No change in chromatin structure, characteristic of patients with Down's syndrome, was demonstrated in the group of fathers and male sibs. Since this phenomenon did not show any correlation between probands, their fathers, and male sibs in the analysis of family cases, we suggest that such a change in the hereditary substrate structure may be a characteristic of the female genotype only.

Discussion

A study has been made of the interphase chromatin structure of lymphocytes in healthy subjects, patients with Down's syndrome, and their parents and sibs by studying thermally induced changes in acridine orange fluorescence of chromatin. The results show that in 40% of healthy normal subjects,

irrespective of the sex of the subject examined, a clear picture is obtained. The changes in lymphocyte chromatin fluorescence with temperature show a regularly repeated pattern made up of a curve with six or seven maxima at different temperatures. In the remaining 60%, various regularly repeated deviations are observed and are correlated with the sex of the subject. There are five subgroups among females and seven subgroups among males.

For patients with Down's syndrome, however, 97% of the lymphocyte chromatin fluorescence profiles give curves with maxima at only three temperatures: 65, 85, and 92°C ($\pm 2^{\circ}C$). Maxima at 78°C and 45°C are absent. Furthermore, 80% of the mothers of probands with Down's syndrome and 30% of their female sibs also show maxima at these three temperatures. Male sibs and the fathers of Down's syndrome probands, however, show fluorescence profiles which do not differ from those of normal subjects.

We believe that, since the changed chromatin

structural organisation we have observed in our experiments is only revealed in the mothers and some female sibs of Down's syndrome patients, there may exist a certain group of subjects within the female population whose genotypical peculiarities are related to the aetiology of trisomy 21.

The nature of the thermally induced chromatin changes revealed by acridine orange (AO) in our studies are, however, not entirely understood. The ratio of red to green acridine orange fluorescence has been widely used as a measure of DNA denaturation to melting,³ but in the present study this ratio remained constant and only the total fluorescence varied. The phenomenon observed does not, therefore, appear to represent actual denaturation of DNA. Displacement of proteins from DNA at higher temperatures, thereby unmasking dye-binding groups, could lead to increased AO binding and fluorescence, but this does not explain the decreases in fluorescence which were also observed. The true explanation of the phenomena observed must therefore await the results of further work. Meanwhile, it must be noted that, whatever the explanation, the thermally induced chromatin

changes revealed by AO fluorescence are consistent within the different classes of subjects studied and are believed to represent some characteristic of the chromatin organisation in these classes.

If there is a group of high risk women who can be ascertained simply by AO fluorescence of their lymphocyte chromatin, then this technique could make an important contribution to the counselling of the female population in respect of their likelihood of producing a Down's syndrome child.

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