STRUCTURE OF INTERPHASE CHROMATIN IN PATIENTS WITH DOWN'S SYNDROME AND THEIR MOTHERS

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Features of an altered structure of the interphase chromatin characteristic of their affected children were found in mothers of children with Down's syndrome by luminescence microscopy of short-term cultures of lymphocytes stained with acridine orange. Abnormalities similar to the changes found in their mothers also were found in girl sibs. The results suggest that there is a certain population of women whose genotype is such that it favors the appearance of this type of chromosome pathology. Since the altered structural organization of the chromatin was found only in the mothers and girl sibs, it seems likely that these features of the genotype are inheritable and are linked with genes (or particular regions of chromatin) limited by sex.

KEY WORDS: *Down's syndrome; chromatin; lymphocytes.*

Several reports have now been published which indicate that certain clinical [6, 7], biochemical [4], immunological [3], and cytogenetic [5] indices characteristic of patients with Down's syndrome can also be found in their phenotypically normal relatives.

Changes in the structure of the interphase chromatin of peripheral blood lymphocytes of patients with Down's syndrome were discovered previously [2] by luminescence microscopy after staining with acridine orange (AO).

The object of this investigagion was to study the structure of the interphase chromatin of lymphocytes from parents of children with Down's syndrome and also from their healthy children.

EXPERIMENTAL METHOD

Interphase chromatin of peripheral blood lymphocytes was studied by luminescence microscopy with the use of AO [8]. Short-term cell cultures were used, i.e., cells incubated in Eagle's nutrient medium with 10% autologous serum for 1 h. A comparative analysis was made of the intensity of fluorescence of the dye after incubation of the lymphocytes for 5 and 60 min and also after 60 min of incubation of cells treated with phytohemagglutinin (PHA).

In parallel tests some of the cells from the same subjects were subjected to comparative analysis based on melting curves of DNA in the composition of the cell chromatin, by the writers' modification [1] of Ringertz's [9] method. Readings were obtained at intervals of 1°C. The change in chromatin structure in response to the action of temperature also was tested with respect to the quantity of bound fluorescent label. The intensity of luminescence of AO bound with the DNA of the lymphocyte chromatin was measured on an MS P-0.5 scanning microscope-photometer (Opton). The wavelength of the exciting light was λ = 365 nm. The intensity of luminescence was measured at λ = 530 nm by means of a suitable interference filter. The apparatus, the details of the technique, and also the isolation and cultivation of the lymphocytes were all described previously [2].

For convenience of analysis of the results, the subjects as a whole were divided into the following groups: 1) healthy, 45 persons (25 women aged from 18 to 35 years, 20 men aged

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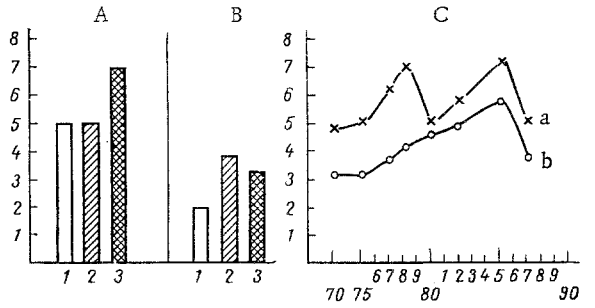

Fig. 1

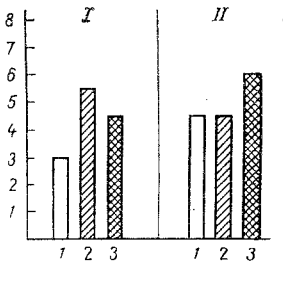

Fig. 2

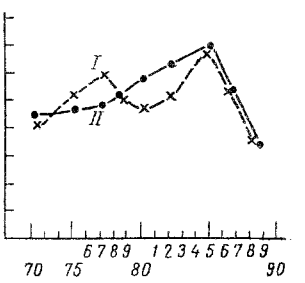

Fig. 3

Fig. 1. Intensity of fluorescence of AO bound with chromatin of human peripheral blood lymphocytes (λ = 530 nm). A) Healthy human lymphocytes; B) lymphocytes of patients with Down's syndrome: 1) after incubation of cells for 5 min; 2) for 60 min; 3) for 60 min in the presence of PHA; C) melting curves of DNA chromatin of human peripheral blood lymphocytes, obtained by luminescence microscopy with AO (F_{530}) within the temperature ranged 70-90°C: a) healthy human lymphocytes; b) lymphocytes of patients with Down's syndrome. Abscissa: C) temperature (in °C); ordinate: A and B) intensity of fluorescence (F); C) ratio of intensity of fluorescence of AO bound with chromatin of human peripheral blood lymphocytes (λ = 530 nm) at temperature T°C to that at 20°C: $F_{530}T°/F_{530}20°$.

Fig. 2. Intensity of fluorescence of AO bound with chromatin of peripheral blood lymphocytes of mothers of children with Down's syndrome. I) Subgroup 1; II) subgroups 2 (see text); 1) intensity of fluorescence after incubation of cells for 5 min; 2) for 60 min; 3) for 60 min in the presence of PHA. Ordinate, intensity of fluorescence of AO (F_{530}).

Fig. 3. Melting curves of chromatin DNA of peripheral blood lymphocytes of mothers of children with Down's syndrome, obtained by luminescence microscopy with AO. I) Subgroup 1; II) subgroup 2 (see text). Abscissa, temperature (in °C); ordinate, ratio of intensity of fluorescence of AO bound with chromatin of human peripheral blood lymphocytes (λ = 530 nm) at temperature T°C to that at 20°C: $F_{530}T°/F_{530}20°$.

from 22 to 42 years); 2) patients with Down's syndrome (probands), 25 persons (15 males and 10 females aged from 18 to 22 years); 3) parents of mongoloid children (35 mothers aged from 20 to 48 years and 15 fathers aged from 28 to 58 years); 4) sibs (12 girls aged from 6 to 22 years and 10 boys aged from 10 to 24 years).

EXPERIMENTAL RESULTS AND DISCUSSION

Healthy Women (group 1). As Fig. 1A shows, after incubation of the cells for 5 and 60 min in most experiments the intensity of fluorescence of the dye was unchanged. Addition of PHA to the incubation medium caused a significant increase in the quantity of dye bound by the cells, by 30-60%. In response to the action of heat the lymphocytes of the healthy donors gave a significant increase in the intensity of fluorescence of the dye by 2-2.5 times at temperatures of 45, 65, 78, 85, 88, and 92°C, i.e., six maxima were regularly obtained ($P < 0.01$; Fig. 1C).

Identical results, indistinguishable from normal, were obtained with the lymphocytes of fathers of children with trisomy 21 (47XY + 21; 47XX + 21), healthy boy sibs, and male blood donors.

Patients with Down's Syndrome (group 2). As Fig. 1B shows, in most experiments the intensity of fluorescence of the dye was low after incubation of the cells for 5 min (compared with the control), and this was interpreted as an increased degree of condensation of the chromatin in patients with regular trisomy. There was also an increase in the intensity of fluorescence of the dye after incubation of the cells for 60 min, i.e., the phenomenon known as spontaneous activation, absent in normal cells, appeared. The mechanism of this phenomenon is not quite clear. Finally, no activating action of PHA could be found after incubation of the cells for 1 h.

In response to the action of heat on the trisomic cells the intensity of fluorescence was regularly increased by 1.5-2 times at temperatures of 65, 85, and 92°C, i.e., three maxima were discovered (Fig. 1C).

The main differences between the DNA melting curves of chromatin of normal and trisomic cells occur within the temperature range of 75-85°C (Fig. 1C). Within this temperature range the curve for trisomic cells rose steadily with one ill-defined maximum in the region of 85°C. The peak at 78°C was absent. It can be concluded from the analysis of the experimental data that in regular trisomy there is a great degree of condensation of the chromatin.

Mothers of Children with Down's Syndrome (group 3). All the subjects of this group could be divided into two subgroups depending on the character of the changes in the chromatin complex (Figs. 2 and 3). Subgroups 1 included persons (21) whose lymphocytes gave a low intensity of fluorescence after incubation of the cells for 5 min (compared with the control), an increase in the intensity of fluorescence of the dye after incubation of the cells for 60 min (the phenomenon known as spontaneous activation occurred), and absence of the activating action of PHA after incubation for 1 h. These changes were characteristic of their children with Down's syndrome (Fig. 2, I).

Meanwhile, in response to the action of heat, the maternal lymphocytes of this subgroup gave a significant increase in the intensity of fluorescence of the dye by 2-2.5 times at temperatures of 45, 65, 78, 85, 88, and 92°C ($P < 0.01$), i.e., six maxima regularly appeared, as is characteristic of healthy human lymphocytes (Fig. 3, I).

Persons for whom the intensity of fluorescence of the dye was unchanged after incubation of their cells for 5 and 60 min (14 people) were placed in subgroup 2. In response to the action of PHA the quantity of dye bound by the cells increased by 30-60%, i.e., the characteristic picture for healthy human lymphocytes was observed (Fig. 2, II).

The action of heat on the lymphocytes from the subjects of this subgroup caused a regular increase (by 1.5-2 times) in the intensity of fluorescence at 65, 85, and 92°C ($P < 0.01$), i.e., three maxima were observed, and this was characteristic of their probands (Fig. 3, II). The main differences between the two subgroups of mothers were obtained within the temperature range from 70 to 90°C. An identical distribution into two subgroups also was discovered among the healthy daughters (subgroup 1 — six individuals, subgroup 2 — four individuals). The results obtained with sibs born before and after the proband were indistinguishable.

In women having children with Down's syndrome certain features of a modified structure of the interphase chromatin characteristic of their affected children were thus discovered. In girl sibs (10 of 12 cases) abnormalities similar to changes found in their mothers also were observed.

In the writers' opinion these results suggest the existence of a certain population of women whose genotype has features that contribute to the appearance of this form of chromosomal pathology.

Since an altered structural organization of the chromatin was found only in the mothers and girl sibs, this suggests that these features of the genotype are inheritable and are linked with genes (or certain regions of chromatin) limited by sex.

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