Satellite Association and Trisomy in Thyrotoxicosis*

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Satellite association has been suggested as having an aetiological role in the development of chromosomal abnormalities (Ferguson-Smith and Handmaker, 1961; Ohno, Trujillo, Kaplan, and Kinoshita, 1961; Ferguson-Smith, 1964). In a study corroborating this theory, some of the unaffected parents of subjects with translocation trisomy-21 were found to have an increased percentage of cells exhibiting satellite association (Zellweger, Abbo, and Cuany, 1966).

Hyperthyroidism in the mother at the time of conception has been suggested as predisposing the foetus to trisomy (Ek, 1959; Fischer, Griffin, England, and Pasamanick, 1961; van Wijck, Stolte, van Kessel, and Tijdink, 1961; Merz, Prempre, and Migeon, 1966). In the latter study, a human testicular tumour was cultured *in vitro* with added L-thyroxine, and the average number of nucleoli per cell was reduced. This suggested to the authors that thyroxine caused these organelles to coalesce, and they speculated that this might result in increased satellite association which could possibly infer increased non-disjunction. However, another *in vitro* study (Siegel and Tobias, 1966) revealed an increased number of nucleoli with thyroid hormone.

Since *in vitro* results were conflicting, and the effect of hyperthyroidism on satellite association in cells *in vivo* had not been clarified, it was examined in thyrotoxic females to determine whether there was any difference from the normal occurrence.

Material and Methods

Blood was obtained from 9 untreated hyperthyroid women with diffuse thyroid enlargement (Table I), and from 9 female controls consisting of laboratory and secretarial workers, and grown in a similar manner for

72 hours at 37° C. Each culture consisted of 1.5 ml. WBC rich plasma, 1 ml. foetal calf serum, 7.3 ml. MEM-Eagle (Flow Labs), and 0.2 ml. PHA-M (Difco) placed in a sterile 30 ml. screwtop medicine bottle. At 69 hours 0.2 ml. colchicine (20 γ per ml.) was added. Cells were treated with 0.9% sodium citrate for 30 minutes, and fixed with two changes of absolute ethanol: acetic acid (2:1). Chromosomes were spread, by blowing on them, allowed to air dry, and stained with Giemsa. Specific criteria for evaluating satellite association were followed, similar to those used by Ferguson-Smith and Handmaker (1961), and Zellweger *et al.* (1966).

Results

Results are based on an average of 100 metaphase spreads per subject. In the 9 hyperthyroid patients, the incidence of cells with satellite association ranged from 21 to 65% (mean 38.3%). The incidence for the 9 controls ranged from 23 to 66% (mean 43.5%). By dividing all the associated acrocentric chromosomes by the total number of acrocentrics examined in each subject multiplied by 100, we obtain the Association Index, expressed as a percentage. This is a more reproducible and more

TABLE I
CONFIRMATORY TESTS IN HYPERTHYROID
PATIENTS OF THYROID OVERACTIVITY AT
TIME LEUCOCYTES WERE OBTAINED

Case No.	PBI (μ g./100 ml.)	¹³¹ I 24-hr. Uptake (%)
1	15	66
2	18	61
3*	17.6	†
4	17.3	58
5	14	83
6	22	72‡ (6-12)
7	17	66
8	10.4	82
9	10.2	13.6‡ (0-4)
Normal	4-8	< 40

* 3 months' pregnant, delivered normal child.

† Free T₄ 5.7 (1.4-2.5 μ g./100ml.); T₄ by column 10.9 (2.9-6.4 μ g./100ml.); Thyroid-binding globulin 23.5 (10-26 μ g./100ml.).

‡ 2-hour ¹³¹I uptake (normal value in parenthesis).

Received June 9, 1967.

* Published in abstract form American Federation for Clinical Research, April 1967. (Atlantic City.)

TABLE II
AGE, PERCENTAGE OF CELLS WITH SATELLITE ASSOCIATION, AND ASSOCIATION INDEX (AS PERCENTAGE)
IN EACH SUBJECT

Controls				Hyperthyroid			
Case No.	Age (yr.)	% Cells with Satellite Association	Association Index as Percentage	Case No.	Age (yr.)	% Cells with Satellite Association	Association Index as Percentage
1	18	49	13.5	1	19	21	4.7
2	19	50	11.2	2	20	44	11.3
3	21	23	4.9	3*	20	37	8.5
4	21	53	13.5	4	21	33	8.8
5	23	66	19.0	5	22	22	5.4
6	23	53	15.7	6	31	64	18.3
7	23	23	5.4	7	32	26	6.8
8	41	44	10.2	8	50	65	17.3
9	46	31	7.1	9	60	33	6.9
Mean		43.5†	11.17†			38.3†	9.76†

* 3 months pregnant, delivered normal child.

† Differences not statistically significant (p approximates 0.5 in each).

$\frac{\text{Associated acrocentrics}}{\text{Total acrocentrics}} \times 100 = \text{Association Index.}$

accurate way to calculate association than the percentage of cells showing satellite association, since some cells have several chromosomes associated. The association index ranged from 4.7 to 18.3% (mean 9.76%), and from 4.9 to 19.1% (mean 11.17%) in the thyrotoxics and normals, respectively (Table II). The difference between the two groups is not significant when calculated on the basis of cells exhibiting satellite association or association index (p approximates 0.5 in each).

Discussion

The study reported here does not reveal any difference in satellite association between hyperthyroid and normal women. Our data for controls compare favourably with those found by Ferguson-Smith and Handmaker (1961). These findings do not exclude the possibility that there is a correlation between thyrotoxicosis and trisomy. Siegel and Tobias (1966) have shown that the addition of thyroxine *in vitro* decreases the time required for cells to double, and also increases the rate of production of nuclear protein and RNA. The latter was reported additionally by Tata (1966). It could be that mitosis and meiosis proceed more rapidly in hyperthyroidism, or that spindle formation and function is not as good as that in normals, and that satellite association has a greater chance of causing non-disjunction and translocation. Another possibility has been raised correlating autoimmune disease with trisomy-21 (Mellon, Pay, and Green, 1963; Fialkow, 1966). Graves' disease is frequently associated with long-acting thyroid stimulator, an immunoglobulin (McKenzie, 1965; Beall and Solomon, 1966; Carneiro, Dorrington, and Munro, 1966). It is thought that this illness could be autoimmune in

nature. Perhaps an immunological defect is a predisposing factor to trisomy in this common form of thyroid overactivity?

Summary

Satellite association occurs in normal and hyperthyroid females with equal frequency.

The authors wish to express their thanks to Drs. T. Merz, W. Mills, W. Leach, and W. Moore, for advice and suggestions; to Dr. J. Whang and Mrs. T. Knudsen, for aid with technical problems; and to Mr. S. Brecher and Mrs. D. Dolimpio, for laboratory assistance.

The authors wish to thank Dr. Phil Gordon of the National Institutes of Health, Bethesda, Maryland, for patients 2, 3, 4, and 8, and Drs. Robb Moses and Turner Bledsoe of the Johns Hopkins Hospital, Baltimore, Maryland for patient 6. The other 4 patients were seen by J. S. or M. B. at their hospitals.

Dr. Gordon Jessup of the National Center for Radiological Health, Rockville, Maryland, kindly performed the statistics, using the standard t test.

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