AUTOIMMUNITY IN WOMEN WITH SEX CHROMOSOME ANEUPLOIDY AND IN THEIR PARENTS COMPARED TO CONTROLS

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SUMMARY

The incidence of thyroid and gastric antibodies in patients with clinical and cytogenetic features of gonadal dysgenesis, in doubly chromatin positive women and in the parents of patients with gonadal dysgenesis was not found to be significantly higher than in controls matched for age.

The incidence of antibodies appeared to be appreciably higher in mosaics and in mosaics whose sex chromosome complement included an isochromosome of the long arm of the X. Failure to establish the significance of these raised incidences may be due to inadequate numbers.

The discrepancy between the findings in this series and in previously reported series is discussed.

INTRODUCTION

In 1961, Engel & Forbes reported a patient with the clinical features of Turner's syndrome with an XX sex chromosome complement, one X being an isochromosome (46,XXqi), who had previously suffered from and been treated for Hashimoto's disease of the thyroid. Two similar cases were reported by Sparkes & Motulsky (1963) and another by Grumbach & Morishima (1964). In the same year Williams, Engel & Forbes (1964) reported a series of twenty-five patients who had ovarian dysgenesis and a variety of sex chromosome abnormalities of whom three had evidence of chronic thyroiditis and thirteen had positive tests for circulating thyroid antibodies. Thyroid antibodies were also frequently found in close relatives of these patients by Fialkow (1966) and more recently by Vallotton & Forbes (1967) who extended the studies on the series first described by Engel & Forbes (1965). Doniach, Roitt & Polani (1968) have also reported that patients with ovarian dysgenesis and abnormal sex chromosomes may be particularly prone to develop thyroid antibodies and also concluded that different chromosomal subgroups differ in their propensities to antibody production. The parents of the patients did not, however, appear to have a raised incidence of antibodies in relation to their ages. In this paper we report on the findings of

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thyroid, gastric and other organ-specific and non-organ-specific antibodies among patients with gonadal dysgenesis and among women with a 47,XXX sex chromosome complement. When the parents of patients with gonadal dysgenesis were alive and accessible for investigation, these too were studied. The frequency of thyroid and gastric antibodies in these subjects has been compared with the results obtained by the same laboratory among healthy blood donors of the same age and sex.

PATIENTS STUDIED

The patients had all been referred to the Registry of Abnormal Karyotypes at the Clinical and Population Cytogenetics Research Unit in Edinburgh. Their age distribution in decades is given in Table 1. Fifty-one of the patients had gonadal dysgenesis (Turner's syndrome) and fourteen were doubly chromatin positive on examination of buccal smears.

Gonadal dysgenesis

This diagnosis was based on a history of amenorrhoea, failure to develop secondary sexual characteristics at puberty, short stature and an abnormal sex chromosome complement compatible with this diagnosis. Of the fifty-one patients, thirty-nine had been identified when they presented with primary amenorrhoea, eight had been referred for investigation of failure to grow, two for investigation of suspected thyroid disturbances, one for secondary amenorrhoea and one for obesity.

Twenty-one of these patients had a 45,X karyotype and twenty-four had a chromosome mosaicism in which one cell line had a 45,X karyotype. The karyotypes of these patients is shown in full in Table 1. The structural abnormalities and the count distributions are described elsewhere under the patients' case numbers in the Registry of Abnormal Karyotypes (Court Brown *et al.*, 1964; and to be published) given in a footnote to Table 1.

None of the fifty-one patients had clinical evidence of thyroid disease at the time of this investigation. However, one had been diagnosed as suffering from thyrotoxicosis (PBI = 13·4 μg/100 ml) when she was originally referred for chromosome studies, since when she has been successfully treated with anti-thyroid drugs for 18 months. Eight of the fifty-one patients had been suspected of suffering from hypothyroidism including seven who had been treated with thyroid extract but in one instance only was the diagnosis confirmed on re-investigation and was disproved in the remainder. Another patient had a thyroglossal cyst excised in childhood. In two patients (108/61; 34/63) the levels of protein bound iodine (9·2 and 11·2 μg/100 ml) were above the upper limit of normal set by the laboratory. The ^{131}I uptakes at 6 hr were 52 and 31%, respectively. Neither patient had clinical evidence of thyroid disease and in both patients the PB^{131}I levels were less than 0·4% of administered dose at 48 hr. In the remaining thirty-one patients whose PBI was estimated, the values (4·0–8·2 μg; mean 6·4 μg/100 ml) were within the normal limits.

Doubly chromatin-positive females

Fourteen women included in the study had been identified as doubly chromatin positive during the routine examination of buccal mucosal smears obtained from patients attending general hospital clinics and from patients at mental subnormality hospitals. In eleven of these, the karyotype was 47,XXX and three were mosaics (Table 1). Seven patients were

mentally retarded and one of these patients had a large firm diffuse goitre and had been given thyroid replacement therapy for several years. Among the remaining patients one is

TABLE 1. Chromosome constitution and ages of fifty-one females with evidence of ovarian dysgenesis and fourteen females who were doubly chromatin positive

Chromosome karyotype	Age (years)						Total
	20	20–29	30–39	40–49	50–59	59	
Gonadal dysgenesis							
45,X	3	12	6	0	0	0	21
46,XXqi	0	5	0	0	0	0	5
46,XXp–	1	0	0	0	0	0	1
Mosaics							
45,X/46,XX	0	1	1	0	0	0	2
45,X/46,XX/47,XXX	0	0	0	0	0	1	1
45,X/46,XY	1	3	0	0	0	0	4
45,X/47,XYY	0	0	0	1	0	0	1
45,X/46,XXqi	2	6	1	1	0	0	10
45,X/46,XXqi/47,XXqiXqi	1	0	0	0	0	0	1
45,X/46,XXr	1	1	0	0	0	0	2
45,X/46,XXmar	0	2	0	0	0	0	2
45,X/46,Xr+	0	0	0	1	0	0	1
Doubly chromatin positive females							
47,XXX	1	3	2	2	1	2	11
45,X/47,XXX	0	0	1	0	1	0	2
46,XX/47,XXX	0	0	0	0	1	0	1

Case No. in registry of abnormal karyotypes

45,X	9/59, 16/59, 54/60, 55/60, 111/60, 162/60, 169/60, 108/61, 169/61, 2/62, 40/62, 95/62, 3/63, 116/63, 61/64, 97/64, 152/64, 78/65, 137/65, 145/65, 314/67.
46,XXqi	35/60, 5/63, 34/63, 97/63, 90/64.
46,XXp–	230/66.
45,X/46,XX	74/61, 154/64.
45,X/46,XX/47,XXX	87/66.
45,X/46,XY	65/62, 59/64, 179/64, 118/65.
45,X/47,XYY	152/60.
45,X/46,XXqi	2/59, 41/60, 168/61, 179/61, 100/62, 101/63, 10/64, 143/65, 188/66, 240/67.
45,X/46,XXqi/47,XXqiXqi	62/63.
45,X/46,XXr	98/65, 140/65.
45,X/46,XXmar	95/64, 42/60.
45,X/46,Xr+	229/66.
47,XXX	55/63, 177/64, 54/66, 52/66, 128/64, 124/64, 51/65, 7/65, 5/65, 259/67, 210/67.
45,X/47,XXX	6/65, 99/66.
46,XX/47,XXX	180/64.

said to have suffered from thyrotoxicosis (confirmatory data not available) and is at present being treated with antithyroid drugs.

Parents of patients with gonadal dysgenesis

Twenty-seven mothers (age range 38–69, mean age 53) and twenty-four fathers (age range 40–77, mean age 55) of patients with gonadal dysgenesis were studied. One of the mothers had suffered from thyrotoxicosis and been treated by partial thyroidectomy and two had diffuse thyroid enlargement. None of the fathers examined had clinical evidence of thyroid disease.

TABLE 2. Incidence of thyroid and gastric antibodies in healthy controls

Age	No. of subjects	Thyroid antibodies						Gastric parietal cell antibodies		Total	
		Thyroglobulin*		Cytoplasmic†		Total					
		No.	%	No.	%	No.	%	No.	%	No.	%
Females											
10–19	53	1	1·9	1	1·9	2	3·8	2	3·8	3	5·7
20–29	87	7	8·0	3	3·4	9	10·3	1	1·1	8	9·2
30–39	78	5	6·4	8	10·0	9	11·5	7	8·9	15	19·2
40–49	70	6	8·0	11	15·7	14	20·0	6	8·0	17	24·3
50–59	70	12	17·0	16	22·8	10	28·5	7	10·0	22	30·1
60–69	46	7	15·2	7	15·2	11	23·9	6	13·0	14	30·4
Total	404	38	9·4	46	11·4	65	16·1	29	7·2	79	19·6
Males											
10–19	47	Nil	Nil	2	4·3	2	4·3	2	4·3	3	6·4
20–29	75	3	4·0	Nil	Nil	3	4·0	2	2·7	4	5·3
30–39	74	2	2·7	Nil	Nil	2	2·7	Nil	Nil	2	2·7
40–49	84	2	2·4	6	7·1	7	8·3	8	9·5	14	16·7
50–59	58	1	1·7	3	5·2	3	5·2	Nil	Nil	3	5·2
60–69	38	1	2·6	Nil	Nil	1	2·6	1	2·6	2	5·3
Total	376	9	2·4	11	2·9	18	4·8	13	3·5	28	7·4

* TCH titre ≥ 1 : 25.
† Positive immunofluorescent staining ± positive complement fixation test.

Controls

The control series consisted of 404 women and 376 men aged between 10 and 69 years. Those between the ages of 20–59 were attending blood transfusion donor sessions. Those at the extremes of the age range were hospital control subjects who had no clinical evidence of thyroid disease, pernicious anaemia, adrenal insufficiency, hypoparathyroidism, diabetes mellitus or any history suggestive of a gross defect of gonadal function and who had no gross growth defect. The age distribution by decades and the numbers with antibodies is given in Table 2.

METHODS

The sera of the patients were examined for antibodies to thyroglobulin by the tanned cell haemagglutination test (TCH) (Fulthorpe *et al.*, 1961) with serum dilutions of 1:5, 1:25 and serial ten-fold dilutions thereafter. Titres ≥1:25 were regarded as positive. Antibody to thyroid cytoplasmic antigen and antibody to the cytoplasm of gastric parietal

cells were examined by the indirect immunofluorescence test using unfixed sections of human thyrotoxic thyroid tissue and gastric mucosa from the body of human stomach using undiluted serum and anti IgG conjugated with fluorescein isothiocyanate (Irvine, 1963; Irvine, Stewart & Scarth, 1967). The method of preparation and properties of the conjugate are described elsewhere (Irvine, Chan & Williams, 1969). Sections of rat kidney were included in each test as a control for specificity and, in particular, for the detection of mitochondrial antibodies (Doniach *et al.*, 1966) and as a further check for antinuclear factors. Tests giving positive immunofluorescence with thyroid or gastric sections were tested and titrated by the method of complement fixation using doubling dilutions of serum starting at 1:2, Takatsy plates, 2 MHD of complement and 50% haemolysis as end point (Irvine, 1966). The immunofluorescence sections were also checked for the presence or absence of nuclear staining.

The sera of forty-nine of the patients with gonadal dysgenesis and of the fourteen women who were doubly chromatin positive were also tested against unfixed cryostat sections of human and rabbit ovary in the follicular and in the luteal phase, human and rabbit testis, human placenta and human adrenal (Irvine *et al.*, 1968, 1969).

RESULTS
Gonadal dysgenesis
Thyroid antibodies

Of the fifty-one patients with clinical and cytogenetic features of gonadal dysgenesis, eight had antibodies to at least one thyroid constituent. This is an incidence of 15·7% as compared with an incidence of 17·0% among 305 controls. Forty-seven of the patients were under the age of 40 years and of these seven had thyroid antibodies. This is an incidence of 14·9% which is higher than the incidence of 10·9% in control women aged 20–39 years but the difference is not significant.

Two of the twenty-one patients with a 45,X karyotype (9·6%) had thyroid antibodies which is not significantly different from controls in the same age group.

Of the twenty-four patients of all ages with mosaicism, six (25%) had thyroid antibodies and, of the twenty who were under 40 years, five (25%) had thyroid antibodies. Both incidences are higher than among female controls (16·1 and 9·2%, respectively) but the differences are not significant. All six patients with thyroid antibodies had a 45,X cell line and three of them also had a 46,XXqi cell line. The three thyroid antibody patients with a 46,XXqi cell line were found among a total of eleven patients who were 45,X/46,XXqi mosaics, an incidence of 27·1%. The other three mosaics who were positive for thyroid antibodies were constituted as follows: 45,X/46,XX; 45,X/46,XY and 45,X/46,X+mar, the second cell line being different in each case. The finding of three patients with thyroid antibodies out of eleven with a 45,X/46,XXqi constitution (27·1%) was not significantly different than the findings among the controls. Finally, no patient was found to have thyroid antibodies out of the five studied whose sex chromosome complement was 46,XXqi without mosaicism.

The tanned red cell haemagglutination titre did not exceed 250 in any of the patients with antibodies. This was also the highest titre among controls under the age of 40 but there were three control women over this age who had titres greater than 250. The highest complement fixation titre of cytoplasmic antibodies among all the patients and among controls under 40 years was 32, but seven of the 305 control subjects over the age of 40 years had titres greater than 32.

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Gastric antibodies

Three of the fifty-one patients with gonadal dysgenesis (5·9%) had gastric parietal cell antibodies as compared with 7·2% of the female controls. The three patients were between 20–33 years of age so that the incidence is 6·4% as compared with 4·8% of the female controls aged 20–39 years. This difference is not significant. The karyotypes of the patients with gastric parietal cell antibodies is shown in Table 3 but the numbers are too small to consider further analysis. The complement fixation titres on the three patients did not exceed 1:32.

The sera of forty-nine of the fifty-one patients were tested for intrinsic factor antibody I and all found to be negative.

Ovarian, testicular, placental and adrenal antibodies

The sera of forty-nine of the patients with gonadal dysgenesis gave negative results for antibodies to ova, theca interna, interstitial cells and corpus luteum of ovary and interstitial cells and spermatids of testis, as well as negative results with placental and adrenal tissue. The sera from the remaining two patients were not available for testing for these antibodies.

Mitochondrial antibody and antinuclear factors

The sera of forty-nine of the patients with gonadal dysgenesis were negative for mitochondrial antibody and for antinuclear factors. The sera of the remaining two patients were not available for this purpose.

Doubly chromatin positive females

Two of the fourteen patients who were doubly chromatin positive had thyroid antibodies and one of these had gastric antibodies. Both patients were over the age of 40 and one was identified when attending a hospital for treatment of hyperthyroidism. The other patient, a 45,X/47,XXX mosaic, had clinical thyroiditis and a TRC titre of 2500. This incidence of antibodies among doubly chromatin females is not significantly raised. No antibodies to ovarian, testicular, placental or adrenal tissue were detected in these patients.

The results of thyroid and gastric antibody tests are detailed in Table 3 and summarized in Table 4.

Parents of patients with gonadal dysgenesis

Thyroid antibodies

Of the twenty-seven mothers examined, five had circulating thyroid antibodies, an incidence of 18·5% which compares with an incidence of 18·7% in control women aged 40–69 years. Two of these with antibodies were the mothers of patients with a 45,X karyotype and three were the mothers of patients whose karyotype included a 46,XXqi cell line. One of the daughters with a 45,X karyotype also had thyroid antibodies.

Of the twenty-four fathers examined, one had thyroid antibodies. His daughter who was a mosaic with a 46,XXqi cell line had no antibodies and neither had her mother. An incidence of one in twenty-four males (4·2%) of this age is lower than in the male control subjects aged 40–69 (6·1%) or in the population study reported by Dingle *et al.* (1966).

TABLE 3. Subjects with positive thyroid and gastric antibody tests

Type	Registry No.	Age	Chromosome complement	Thyroid antibodies			Gastric parietal cell antibody	
				Thyroglobulin (tanned red cell titre)	Epithelial cytoplasm		Immuno-fluorescence	Complement fixation titre
					Immuno-fluorescence	Complement fixation titre		
Group 1	16/59	28	45,X	5	+	2	+	2
	2/62	23	45,X	25	+	4	Neg	Neg
	61/64	20	45,X	Neg	Neg	Neg	Neg	32
Mosaics	74/61	24	45,X/46,XX	250	+	8	Neg	Neg
	59/64	22	45,X/46,XY	25	+	32	Neg	Neg
	2/59	41	45,X/46,XXqi	5	+	2	Neg	Neg
	100/62	20	45,X/46,XXqi	250	Neg	Neg	Neg	Neg
	62/63	18	45,X/46,XXqi/47, XXqiXXqi	25	+	Neg	Neg	Neg
	95/64	20	45,X/46,XXmar	20	+	Neg	Neg	Neg
	143/65	33	45,X/46,XXqi	Neg	Neg	Neg	+	16
Doubly chromatin positive females	55/63	46	47,XXX	Neg	+	4	+	16
	6/65	51	45,X/47,XXX	2500	+	64	Neg	Neg

Gastric antibodies

Three of the twenty-seven mothers (11·1%) and none of the fathers had gastric parietal cell antibodies. These findings do not differ from controls in the same age range, 10·2% of the control women and 5% of the control males age 40–69 years having gastric antibodies.

DISCUSSION

In a group of fifty-one patients with clinical and cytogenetic features of gonadal dysgenesis (Turner's syndrome) and in fourteen women whose buccal smears were doubly chromatin positive, the incidence of thyroid and gastric antibodies was not significantly raised above that in normal controls. Antibodies were also not detected more frequently in a group of parents of patients with gonadal dysgenesis. These findings are in striking contrast to those of Vallotton & Forbes (1967) and of Doniach *et al.* (1968) who found the overall incidence of thyroid antibodies amongst patients with gonadal dysgenesis to be raised (51 and 39·7%, respectively).

TABLE 4. Incidence of thyroid and gastric antibodies with different sex chromosome constitution

Karyotype	No. of patients	Thyroid antibodies		Gastric antibodies	
		No.	%	No.	%
45,X	21	2	9·6	2	9·6
46,XXqi	5	0	0	0	0
46,XXp–	1	0	0	0	0
All mosaics	24	6	2·5	1	4·2
Mosaics with XXqi cell line	11	3	27·1	1	9
Doubly chromatin positive females	14	2	18·3	1	14·2
Female controls	404	65	16·1	29	7·2

The patients we studied were not randomly selected samples of patients with Turner's syndrome or of doubly chromatin positive women but we are not aware of any factors in the selection of the patients which would exclude those with thyroid antibodies. Our patients were considerably younger than those of Vallotton & Forbes, the respective mean ages being 26 and 42 years. This could be an important difference as it is well known that the incidence of antibodies increases with age, but when this younger age of our patients is allowed for and the results compared with findings in women under the age of 40, it was still not possible to demonstrate any increase in the incidence of antibodies. Furthermore the patients studied by Doniach *et al.* (1968) were in fact younger than our patients so the discrepancy with their series cannot be attributed to an age difference.

Our patients could be equally divided into those who had presented at endocrine clinics and those who had presented at gynaecology clinics and the incidence of antibodies was the same in patients from both sources. All but four patients were under investigation for primary amenorrhoea or for failure to grow. Many of the patients studied by Vallotton & Forbes (1967), however, were attending hospital for a variety of conditions which may or may not be associated with the chromosomal abnormality (Engel & Forbes, 1965). A higher

incidence of antibodies has been reported amongst women in the hospital populations by Hackett, Beech & Forbes (1960) so that it is possible that the way in which patients with gonadal dysgenesis present may influence the incidence of thyroid antibodies. Unfortunately the mode of presentation of the patients is not recorded by Doniach *et al.* (1968).

Among our patients only the mosaics appeared to have an appreciably higher incidence of thyroid antibodies. Although this increase was not significant, this could be due to insufficient numbers, and it is noteworthy that Doniach and her colleagues also found the highest incidence of antibodies among patients with sex chromosome mosaicism. The largest subgroup of the mosaics we studied had an XXqi cell line and half the antibody positive cases were in this group, whilst the others were distributed among mosaics with a variety of second cell lines. It is of interest that cases which first drew attention to a possible association between autoimmune thyroiditis and Turner's syndrome were remarkable in that they all had a sex chromosome complement which included an isochromosome of the long arm of the X, and it was the possible association with additional X chromosome material that prompted the inclusion of women with XXX sex chromosomes in this survey. It may be significant that the only patient with clinical thyroiditis was in this group.

One of us (Irvine *et al.*, 1968) has reported the incidence of two cases of gonadal dysgenesis with normal chromosome constitution in whom there was a strong association with autoimmune disease; idiopathic adrenal atrophy, idiopathic hypoparathyroidism in the first case and these conditions plus primary atrophic hypothyroidism and pernicious anaemia in the second. Both of these patients had a high incidence of autoantibodies of the organ-specific type in the serum.

The parents of only twenty-seven patients with gonadal dysgenesis were studied but again we are not aware of any circumstances which would lead to the exclusion of those having thyroid antibodies and we cannot account for the difference between the incidences of antibodies in this series and in the parents studied by Vallotton & Forbes (1967). Doniach *et al.* (1968), however, were also unable to show a raised incidence of antibodies in parents of patients with gonadal dysgenesis.

It is difficult to account for the differences between our findings in patients with gonadal dysgenesis and those previously reported, except on the basis of differences in the selection of patients. It is clear that in order to determine the significance of apparently high incidences of antibodies it is important that comparisons be made with controls matched not only for age but also for mode of presentation. However, the relatively high incidence of antibodies to thyroid and gastric mucosal cells in the general population will probably make it necessary to study large numbers in order to establish or exclude significant differences. The negative findings in tests for other organ-specific antibodies do not suggest, however, that there is an increased risk of autoimmunity in general among these patients.

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