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21-hydroxylase autoantibodies are more prevalent in Turner syndrome but without an association to the autoimmune polyendocrine syndrome type I

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Summary

Turner syndrome (TS) is associated with an increased frequency of autoimmunity. Frequently observed autoimmune diseases in TS are also seen in the autoimmune polyendocrine syndrome type I (APS I), of which Addison disease is a key component. An overlapping antibody profile between TS and APS I could be considered. The aim of this work was to study women with TS regarding 21-hydroxylase (21-OH) antibodies and interferon omega (IFN-ω) antibodies, a highly specific marker for APS I, to determine if there are immunological overlaps between TS and APS I. Blood samples from 141 TS were assayed for 21-OH antibodies and IFN-ω antibodies using *in-vitro*-transcribed and translated autoantigen. Indices with a cut-off point of 57 and 200 for 21-OH antibody and IFN-ω antibody were used as reference. The median age of TS was 31·6 years (range = 11·2–62·2). Positive indices of 21-OH antibodies were present in six TS (4%), with a mean of 144·8 (range = 60–535). None had apparent adrenal insufficiency. There was no age difference comparing 21-OH antibody-positive TS (median age = 33·9 years, range = 17·7–44·7) and 21-OH antibody-negative TS (median age = 31·6 years, range = 11·2–62·2) (P = 0·8). No TS was positive for IFN-ω antibodies (mean = 42·4, range = –435–191). No overlapping autoimmune profile between TS and APS I was found. Autoimmunity against 21-OH among TS patients was more prevalent than previously identified, suggesting an increased risk of adrenal failure in TS. However, whether adrenal impairment will develop remains unknown.

Keywords: Turner syndrome, adrenal insufficiency, autoimmune polyendocrine syndrome type

Introduction

Turner syndrome (TS) is caused by complete or partial X chromosome monosomy. Afflicting approximately 1 : 2000 newborn girls, it is one of the most common sex chromosome abnormalities [1]. Cardinal features of TS are short stature, lack of spontaneous puberty and infertility due to ovarian dysgenesis. Autoimmunity has been recognized as one of the more prominent characteristics of TS, and an increasing prevalence with age [2] makes continuous screening for specific autoimmune diseases recommended [3]. Anti-thyroid autoantibodies predisposing to autoimmune thyroiditis with subclinical or overt hypothyroidism is the most frequent autoimmune disease in TS, affecting up to 50% of TS girls and women [2,4]. Other autoimmune diseases occurring more frequently are inflammatory bowel diseases, juvenile rheumatoid arthritis, alopecia, psoriasis and type 1 diabetes mellitus (DM1) [2,5–8]. In contrast, an association between TS and adrenal insufficiency has not been reported.

The underlying cause for the apparent predisposition to autoimmunity in TS remains an enigma. Immunological alterations such as a decreased CD4 : CD8 ratio in TS has been suggested as a factor predisposing to autoimmunity [9,10]. Genetically, haploinsufficiency is a

contributor to autoimmunity, which has also been proposed as a possible explanation for the higher prevalence of male-predominant autoimmune diseases in TS compared to the prevalence of female-predominant autoimmune diseases [11]. A study comparing TS women and women with primary ovarian insufficiency (POI) suggested an association between POI *per se* and autoimmunity [12].

Autoimmune polyendocrine syndrome type I (APS I) is characterized by the development of at least two of three key components during childhood: (1) chronic mucocutaneous candidiasis; (2) hypoparathyroidism; and (3) adrenal failure [13]. Moreover, many ectodermal manifestations are common, notably vitiligo, alopecia, keratitis and enamel dysplasia, all conditions reported more frequently among TS [3]. A variety of autoantibodies are associated with APS I, here among interferon (IFN) antibodies of which IFN-ω antibodies is present in nearly all APS I patients [14–17]. Thus, IFN-ω antibodies serve as a robust screening for APS I.

In the present study, we aimed to study women with TS regarding 21-OH antibodies and IFN-ω antibodies to determine if there are immunological overlaps between TS and APS I.

Materials and methods

Patients

From 2003 to 2008, a total of 141 TS girls and women were recruited from the National Society of Turner Contact Groups in Denmark, the Department of Endocrinology and Internal Medicine at Aarhus University Hospital, the Department of Pediatrics at Hillerød Hospital and the Children's Hospital at Glostrup Hospital. All interested subjects were included. Prior to inclusion, all subjects had been karyotyped. The distribution of karyotypes is presented in Table 1. No controls were included in the study.

Table 1. Distribution of karyotypes in the Turner syndrome (TS) study cohort

Karyotype	Number of TS patients
45,X; 45,X,13s+,14s+,22s+; 45,X,15p+; 45,X,21s+; 45,X,inv(9); 45,X,inv(9)(p11q11); 45,X,9qh+	76
45,X/46,XX; 45,X/46,XX/47,XXX; 45,X/46,XXp+; 45X/47XXX	11
45,X/46,XY, marker(Y); 45,X/46,X,+mar (ring); 45,X/46,X,r(X); 45,X/46,X,+mar;	6
45,X/46,XY; 45,X/46,X,r(Y),var(15)(p13,qfq35)	7
45,X/46,X,i(Yq); 45 X/46 Xi (Xq); or equivalents	35
45,X/46,X,del(X)(p22p21p11); 46,X,del(X)(q22.2); or equivalents	4
45,X/47,XXX	2
Total	141

Methods and assays

A medical history concerning previously diagnosed autoimmune diseases [thyroid disease, adrenal failure, DM1 or coeliac disease (CD)] was obtained by a questionnaire completed by the participant or by interview (adhering to the questionnaire). A venous blood sample was drawn. Blood samples were assayed prospectively.

21-OH antibodies and IFN-ω antibodies were measured using *in-vitro* transcribed and translated radiolabelled autoantigen [17]. Results are expressed as indices [counts per minute (cpm) sample – cpm-negative control/cpm-positive control – cpm-negative control) × 1000]. Pooled human serum from 150 healthy blood donors was used as negative control, and a patient with intermediate 21-OH antibody titre or intermediate IFN-ω antibody titre was used as positive control. The threshold for positivity was set as the mean of 150 negative controls +3 standard deviations, resulting in cut-off points of 57 and 200 for 21-OH antibody and IFN-ω antibody, respectively [17]. The negative controls were blood donors collected completely anonymously at the Blood Bank at Haukeland University Hospital, Norway. The standard number of negative controls used to establish reliable cut-off values are 120 [18], thus the present number of negative controls are above standard. The serological testing for 21-OH antibodies and IFN-ω antibodies was successful in 139 of 141 patients.

Of the entire cohort, 100 patients underwent additional serological testing for immunoglobulin (Ig)A anti-gliadin, IgA anti-transglutaminase, IgG anti-thyroid peroxidase (anti-TPO) and anti-glutamic-acid decarboxylase 65 (anti-GAD-65). In the case of IgA deficiency, anti-gliadin and anti-transglutaminase IgG were measured. If CD-related autoantibodies were detected, patients were referred to endoscopic examination with biopsies to confirm or exclude CD. Anti-TPO and anti-GAD-65 were determined using conventional enzyme-linked immunosorbent assay (ELISA). The serological testing was successful in 99 of 100 patients. These results were published previously as part of a cohort of 107 TS patients [2].

Detection of autoantibodies without a related medical history resulted in contact with the patient (or her parents) and her physician to enable further investigation outside the study setting.

Table 2. Autoimmune diseases in the Turner syndrome (TS) study cohort

Disease	Percentage of TS patients with autoimmune disease	Median age (years, range)
Hypothyroidism	14% (20 of 141)	39·7 (13·5–60·2)
Coeliac disease	1% (2 of 141)	38·4 (26·4–50·4)
Diabetes mellitus type 1	1% (1 of 141)	46·4 (n.a.)
Adrenal failure	0% (0 of 141)	n.a.
Total	16% (22/141)	39·7 (13·5–60·2)

n.a. = not applicable.

Statistics

Data were analysed using Stata version 13.1 for Windows (StataCorp, College Station, Texas, USA). Ages were compared using the Kruskal–Wallis test. Distributions among groups were tested by Fisher's exact test. *P*-values $<0{\cdot}05$ were considered statistically significant.

Ethics

The study was approved by the Aarhus County Committee on Biomedical Research Ethics (no. 20030116) and the Danish Data Protection Agency.

Results

Overall, 16% (22 of 141) of TS patients were diagnosed with one of the autoimmune diseases of interest when included into the study: (i) hypothyroidism: 14% (20 of 141); (ii) CD: 1% (two of 141); and (iii) DM1: 1% (one of 141). No patients (none of 141) had adrenal insufficiency (Table 2), and one patient was diagnosed with two of the diseases of interest: hypothyroidism and DM1.

The median age of the entire cohort was 31·6 years (range = 11·2–62·2). The median age among those diagnosed with at least one disease of interest was higher than the median age among those who had no diagnosis of interest [39·7 years (range = 13·5–60·2) *versus* 30·5 years (range = 11·2–62·2), $P = 0{\cdot}03$].

The mean index of 21-OH antibodies among all TS was 9·3 (range = –84 to 535). Four percent (six of 139) had an index above the positive threshold (mean = 144.8, range = 60–535) (Table 3). There was no difference in age comparing 21-OH antibody-positive patients and 21-OH antibody-negative patients [33·9 years (range = 17·7–44·7) *versus* 31·6 years (range = 11·2–62·2, $P = 0{\cdot}8$]. One TS being 21-OH antibody-positive was also tested positive for IgA anti-gliadin, and one was clinically hypothyroid. However, anti-TPO was not measured in this patient (Table 3).

The distribution of 21-OH antibody positivity among karyotypes was as follows: (i) 45,X: $n = 2$; (ii) isochromosomes: $n = 3$; and (iii) Y chromosome material: $n = 1$ (Table 3). No statistically significant association was found between karyotype and 21-OH antibody positivity (45,X: $P = 0{\cdot}4$; TS karyotype with an isochromosome: $P = 0{\cdot}2$).

Among all TS, none (of 139) had IFN-ω antibodies indices above 200 [mean IFN-ω antibodies = –42.4 (range = –435 to 191)].

Discussion

In the present study, 4% of TS were positive for autoantibodies against 21-OH, thus higher than previously identified among both TS and the general population, while no overlapping autoantibody profile between TS and APS I was demonstrated.

Addison disease has not been reported to occur more frequently among TS than in the general population, although an increased frequency of Addison disease among individuals with primary ovarian failure has been reported [19]. Previously, we and others have reported the prevalence of 21-OH antibodies in TS to be between 0 and 1% [2,7,20], which is fairly comparable to the prevalence reported among healthy subjects [21]. In the present study, 4% of the

Table 3. Details regarding subjects with Turner syndrome being positive for 21-hydroxylase autoantibodies

Patient	Karyotype	Age (years)	21-OH antibody index[a]	Adrenal insufficiency	IFN-ω antibodies[b]	Anti-TPO/ hypothyroidism	Anti-GAD-65/ DM 1	IgA anti-gliadin/ coeliac disease
1	45,X	44·7	66	–	2	– / –	– / –	– / –
2	45,X	30·9	535	–	120	– / –	– / –	– / –
3	45,X/46,X i(Xq)	42·2	73	–	9	– / –	– / –	+ / +
4	46X, idic (x)(q)	23·5	60	–	–153	n.a. /+	n.a. / –	n.a. / –
5	45,X/46,X, del(X)(Cenpter)	37	66	–	–49	– / –	– / –	– / –
6	45,X/46,XY	17.7	69	–	–33	– / –	– / –	– / –

[a]Positive threshold for 21-OH antibodies: index = 57. [b]Positive threshold for IFN-ω antibodies: index = 200; anti-GAD-65 = anti-glutamic acid decarboxylase 65; anti-TPO = thyroid peroxidase antibodies; IFN-ω = interferon omega antibodies; DM 1 = diabetes mellitus type 1; n.a. = not applicable; 21-OH = 21-hydroxylase autoantibodies.

subjects had 21-OH antibodies above the positive threshold, although just slightly above the threshold in five of the six subjects. We have no information concerning the level of adrenocorticotrophic hormone (ACTH) and cortisol in these subjects and no synacthen test was performed, but none had a medical history comprising adrenal insufficiency. In a follow-up study on individuals who had no clinical apparent sign of adrenal failure but positive titres of 21-OH antibodies at baseline, 33% (12 of 36) had developed subclinical adrenal impairment or overt adrenal failure after a mean period of 5 years. Whether 21-OH antibody-positive TS subjects in the present study will develop Addison disease remains unknown. Nevertheless, owing to the rarity of Addison disease, it would require a very large cohort to draw any firm conclusion on an association between TS and Addison disease.

Many of the autoimmune diseases frequently observed in TS are seen in APS I as well, hence an overlapping antibody profile could be considered. Such an overlap has been observed between myasthenia gravis and APS I [22]. However, in the present study, IFN-ω antibodies were below the threshold for positivity in all TS subjects, thus in line with previous findings by Stenberg *et al.* among 110 TS girls and women [7] making a putative association between TS and APS I seeming weak. However, very large studies, including probably more than 1000 TS, are needed to clearly delineate frequencies of APS I and presence of IFN-ω antibodies.

As expected, hypothyroidism was the most common autoimmune disorder among TS in the present study, emphasizing the importance of continuous screening of the thyroid function in TS management [3]. We and others have reported DM1 to occur more frequently among TS [5,11,23] than in the general population, whereas others have not [12]. One subject had a diagnosis of DM1 thus, in itself, not supporting an increased risk of DM1. However, as part of a previous study we found that this subject and three additional subjects from the present cohort were GAD-65-positive [2], thus still pointing towards an increased risk of DM1.

In conclusion, no overlapping autoimmune profile between TS and APS I was found. Autoimmunity against 21-OH among TS was more prevalent than previously identified, suggesting an increased risk of adrenal failure in TS. However, none had developed adrenal impairment at the time of the study; thus, further studies are needed.

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Conflict of interest

The authors have nothing to declare.

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