Endocrine
DOI 10.1007/s12020-015-0613-y

ORIGINAL ARTICLE

Screening of endocrine organ-specific humoral autoimmunity in 47,XXY Klinefelter's syndrome reveals a significant increase in diabetes-specific immunoreactivity in comparison with healthy control men

Francesca Panimolle[1] · Claudio Tiberti[1] · Simona Granato[1] · Antonella Semeraro[1] · Daniele Gianfrilli[1] · Antonella Anzuini[1] · Andrea Lenzi[1] · Antonio Radicioni[1]

Received: 14 January 2015 / Accepted: 20 April 2015

Abstract The aim of this study was to evaluate the frequency of humoral endocrine organ-specific autoimmunity in 47,XXY Klinefelter's syndrome (KS) by investigating the autoantibody profile specific to type 1 diabetes (T1DM), Addison's disease (AD), Hashimoto thyroiditis (HT), and autoimmune chronic atrophic gastritis (AG). Sixty-one adult Caucasian 47,XXY KS patients were tested for autoantibodies specific to T1DM (Insulin Abs, GAD Abs, IA-2 Abs, Znt8 Abs), HT (TPO Abs), AD (21-OH Abs), and AG (APC Abs). Thirty-five of these patients were not undergoing testosterone replacement therapy TRT (Group 1) and the remaining 26 patients started TRT before the beginning of the study (Group 2). KS autoantibody frequencies were compared to those found in 122 control men. Six of 61 KS patients (9.8 %) were positive for at least one endocrine autoantibody, compared to 6.5 % of controls. Interestingly, KS endocrine immunoreactivity was directed primarily against diabetes-specific autoantigens (8.2 %), with a significantly higher frequency than in controls ($p = 0.016$). Two KS patients (3.3 %) were TPO Ab positive, whereas no patients were positive for AD- and AG-related autoantigens. The autoantibody endocrine profile of untreated and treated KS patients was not significantly different. Our findings demonstrate for the first time that endocrine humoral immunoreactivity is not rare in KS patients and that it is more frequently directed against type 1 diabetes-related autoantigens, thus suggesting the importance of screening for organ-specific autoimmunity in clinical practice. Follow-up studies are needed to establish if autoantibody-positive KS patients will develop clinical T1DM.

Keywords Klinefelter's syndrome · Type 1 diabetes · Hypogonadism · Autoimmunity

Introduction

Klinefelter's syndrome (KS) is the most frequent sex chromosomal disorder in men [1], occurring in about 150 per 100,000 men. The genetic background for the KS phenotype is the presence of one or more extra X chromosome. About 80 % of cases are due to the 47,XXY karyotype; the remaining 20 % of patients have higher-grade chromosome aneuploidies, mosaicism, or structurally abnormal X chromosome [1].

The clinical presentation of KS is primarily characterized by gynecomastia, small, firm testes, and hypergonadotropic hypogonadism (high gonadotropin, low androgens, and high estrogens) [2]. KS has been associated with thrombosis, varicose veins, type 2 diabetes (T2DM), bone fractures, epilepsy, and neurological and mental disorders [3] with a significant higher comorbidity rate compared to the general male population. Furthermore, there is literature evidence of an increase in both humoral and cellular immunity in male hypogonadism and an increased frequency of autoimmune diseases in KS patients [4]. This may be due to sex hormones, that seem to play an important role as modulators of the onset and/or perpetuation of autoimmune disease. Steroid hormones have been shown to be implicated in the immune response, with estrogens as enhancers of at least humoral immunity and androgens and progesterone as immune suppressors [5–7].

✉ Francesca Panimolle
francescapanimolle@hotmail.it

[1] Section of Medical Pathophysiology, Department of Experimental Medicine, Center of Rare Diseases, Sapienza University of Rome, Viale del Policlinico 155, 00161 Rome, Italy

Published online: 03 May 2015

Table 1 Comparison between KS patients (Group 1 and 2) and control group

Parameters	KS patients ($n = 61$)	KS Group 1 ($n = 35$)	KS Group 2 ($n = 26$)	Control Group ($n = 122$)
Age (years) ± SD range	35 ± 11.4 (18–63)	32 ± 10.7 (18–56)	±37 ± 12.5 (19–63)	41 ± 12.1 (18–65)
Years of follow-up (mean) range	1.5 (0–7)	1.7 (0–7)	1.3 (0–7)	0
TRT mean (years) ± SD	3.0 ± 5.9	0	8 ± 7.4	0
FSH (mIU/ml) ± SD	23.2 ± 17.9	28.5 ± 14.1	15.6 ± 18.9	4.3 ± 1.8
LH (mIU/ml) ± SD	13.2 ± 8.4	15.4 ± 6.7	8.4 ± 8.7	3.2 ± 1.3
T (ng/ml) ± SD	4.8 ± 4.0	5.0 ± 4.9	5.6 ± 2.5	5.9 ± 1.6
E2 (pg/ml) ± SD	31.0 ± 15.9	31.1 ± 16.5	32.2 ± 20.3	29.3 ± 12.6
SHBG (nmol/l) ± SD	45.3 ± 31.8	51.0 ± 33.6	31.3 ± 13.4	34.2 ± 14.1

TRT testosterone replacement therapy, *FSH* follicle-stimulating hormone, *LH* luteinizing hormone *T* testosterone, *SHBG* sex hormone-binding globulin, *E2* estradiol

Even though KS was first described more than 60 years ago and various studies have underlined its concomitant occurrence with autoimmune diseases, our knowledge of KS-related endocrine autoimmunity is still limited and restricted to a few studies [8, 9]. To date, little information is available on any endocrine organ-specific autoimmunity in KS. The aim of our study was therefore to investigate the frequency of the humoral immune response specific to four different endocrine autoimmune diseases, namely type 1 diabetes (T1DM), Addison's disease (AD), Hashimoto thyroiditis (HT), and autoimmune chronic atrophic gastritis (AG), in a cohort of adult Caucasian KS patients with the classic 47,XXY karyotype. We analyzed serum autoantibodies directed against four well-characterized pancreatic islet proteins specific for T1DM [insulin, glutamic acid decarboxylase (GAD), tyrosine phosphatase 2 (IA-2), and islet beta-cell zinc cation efflux transporter (ZnT8)] [10, 11]; against the enzyme steroid 21-hydroxylase (21-OH), the main adrenal autoantigen in AD [12, 13]; against thyroid peroxidase (anti-TPO Ab) characteristic of HT [14]; and against gastric parietal cells (APC Ab) specific to AG [15].

Materials and methods

47,XXY KS patients

A total of 61 adult Caucasian KS patients with the classic 47,XXY karyotype were analyzed (median age 35.0 ± 11.4 years; age range 18–63 years). These patients were recruited from the Center of Rare Diseases, Department of Experimental Endocrinology, "Sapienza" University of Rome from 2007 to 2014. The KS diagnosis was based on clinical features, high levels of follicle-stimulating (FSH) and luteinizing (LH) hormones and low total testosterone serum concentrations (T). The diagnosis was confirmed by karyotype analysis from cultured peripheral lymphocytes. Serum FSH, LH, T, estradiol (E2), and sex hormone-binding globulin (SHBG) concentrations were measured by chemiluminescent microparticle immunoassay technology (CMIA) (Architect System, Abbott). The mean serum levels of the hormones FSH, LH, T, E2, and SHBG were reported in Table 1.

Patients were subdivided and analyzed according to testosterone replacement therapy (TRT):

Group 1 35 KS patients who had never undergone TRT (median age 32 years ± SD 10.7; age range 18–56 years); *Group 2* 26 KS patients undergoing TRT (median age 37 years ± SD 12.5; age range 19–63 years) (Table 1).

Healthy control subjects

Data from KS patients were compared to those from 122 serum samples collected from healthy men (median age 41 years ± SD 12.1; age range 18–65 years). To establish a limit of positivity specific for the adult male population a preliminary study was conducted for each assay, testing sera from 158 healthy men (median age 42 years ± 12.9; age range 18–70 years). The limit of positivity of the methods used in the study was calculated according to the 99th percentile of the values detected in each assay.

All control subjects analyzed had a history of spontaneous puberty with gonadotropin levels within the normal range (Table 1), no clinical evidence of autoimmune diseases and physical parameters within the normal range.

Detection of diabetes-specific autoimmunity

In order to simplify, speed up, and considerably reduce the costs of screening for diabetes-associated humoral autoimmunity, the immune response directed against four well-characterized pancreatic islet proteins specific to autoimmune T1DM (Insulin, GAD, IA-2 and ZnT8) was measured using a single combined fluid-phase radioimmunoprecipitation assay based on the detection of the four autoantibodies in individual assays [novel multi-autoantigen radioimmunoassay (MAA)] [16]. MAA showed 92 %

sensitivity and 99 % specificity in the Diabetes Autoantibody Standardization Program (DASP) held in 2010. Only MAA-positive sera were subsequently tested in single assays to determine individual diabetes-specific humoral immunoreactivities.

Diabetes-specific single autoantibody measurements

Insulin autoantibodies Insulin antibodies were measured using a competitive fluid-phase radioimmunoassay (RIA) [17], with minor modifications. ^{125}I radiolabelled insulin was purchased from Adaltis Italia (RADIM SpA, Italy). This assay achieved 46 % sensitivity and 100 % specificity for insulin antibody detection at the Islet Autoantibody Standardization Workshop (IASP) held in 2012.

GAD autoantibodies GAD antibodies were measured by a previously described RIA fluid-phase method [18] using a human recombinant full-length GAD65 cDNA provided by Dr Å. Lernmark (Lund University, Malmö, Sweden). The GADA assay achieved 80 % sensitivity and 98 % specificity at the Fourth DASP held in 2007 [19].

IA-2 autoantibodies IA-2 antibodies were detected by a RIA fluid-phase method [20] using a human recombinant IA-2 (a.a.605–979) cDNA provided by Dr E. Bonifacio (University of Dresden, Dresden, Germany). The IA-2A autoantibody assay achieved 72 % sensitivity and 99 % specificity at the Fourth DASP [19].

ZnT8 autoantibodies ZnT8 antibodies were detected by a RIA fluid-phase method using human ZnT8 probe (pJH4,1) provided by John Hutton (University of Colorado, Denver, USA) [21]. The ZnT8A assay achieved 58 % sensitivity and 93 % specificity at the standardization program for determination of 21-OH Abs held in 2011 [22].

Detection of adrenal-specific autoimmunity

Autoantibodies against the enzyme steroid 21-hydroxylase (21-OH Abs) were tested by RIA fluid-phase assay using a human recombinant human 21-OH provided by Alberto Falorni (University of Perugia, Perugia, Italy). The 21-OHAb assay achieved 94 % sensitivity and 100 % specificity at the first international serum exchange for the determination of 21-hydroxylase autoantibodies [23].

Detection of thyroid-specific autoimmunity

Thyroid peroxidase autoantibodies (anti-TPO Abs) [24] were measured using Architect® chemiluminescent microparticle immunoassays (CMIA) (Architect System, Abbott Diagnostic Division, United States).

Detection of gastric-specific autoimmunity

Parietal cell autoantibodies (APC Abs) specific for autoimmune chronic AG [25] were measured using a commercially available ELISA kit (Axa Diagnostics, Italy).

Statistical analysis

SAS 9.2 software was used for statistical analysis. Data were expressed as frequencies or as mean ± standard deviation or median values. Frequency differences were calculated by Fisher's exact test. A p value <0.05 was considered significant.

Results

Endocrine organ-specific humoral autoimmunity in 47,XXY KS patients and in healthy men

The overall frequency of organ-specific autoimmunity in our cohort of 47,XXY KS patients and in our male controls was 9.8 % (95 % CI 2.3–17.3) and 6.5 % (95 % CI 2.2–10.8), respectively. Six of 61 KS patients and 8 of 122 controls were positive for at least one of the organ-specific autoantibodies investigated. The distribution of the autoantibody frequencies in our KS patients and control group is reported in Fig. 1.

Diabetes-specific autoimmunity

Five of the 61 47,XXY KS patient serum samples analyzed (8.2, 95 % CI 1.3–15.1) were multi-autoantigen (MAA) positive, with a significantly higher frequency than in the control group (0.8 %, 1/122, 95 % CI −0.8 to 2.4) ($p = 0.0163$).

Subsequently, the MAA-positive sera were tested in single assays to determine individual diabetes-specific humoral immunoreactivities. Three KS cases and one control were positive for just one diabetes-specific autoantibody (GAD Abs, IA-2 Abs or ZnT8 Abs), while the other 2 KS patients were each positive for two autoantibodies (GAD/IA-2 Abs and GAD/Insulin Abs, respectively) (Table 2).

Adrenal-specific autoimmunity

None of the 61 KS patients or 122 controls investigated was autoantibody-positive for 21-OH Abs.

Thyroid-specific autoimmunity

The anti-TPO autoantibody frequency in KS patients and controls was 3.3 % (2/61, 95 % CI −1.2 to 7.8) and 6.5 %

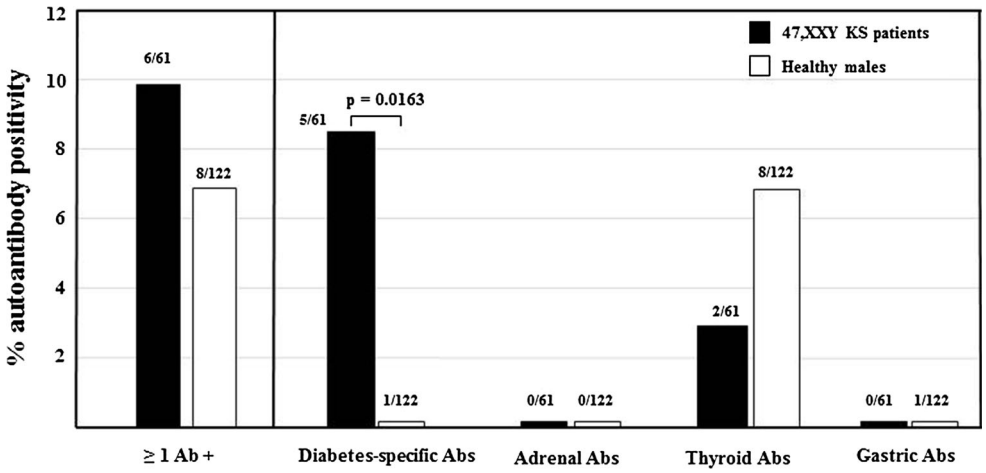

Fig. 1 Frequency of endocrine autoantibody specificities in the 61 47,XXY KS patients and 122 healthy men investigated. Diabetes-specific Abs: glutamic acid decarboxylase (GAD Abs), insulin (Insulin Abs), tyrosine phosphatase (IA-2 Abs), and islet beta-cell zinc cation efflux transporter (ZnT8 Abs) autoantibodies; Adrenal Abs: 21-hydroxylase antibodies; Thyroid Abs: thyroid peroxidase antibodies (TPO Abs); Gastric Abs: autoantibodies to the gastric parietal cell (APC Abs)

Table 2 Immunoreactivity patterns in 47,XXY KS autoantibody-positive patients with no history of previous hormone replacement therapy and in patients undergoing therapy

47,XXX patients	Years	TRT	TRT (years)	MAA	21-OH Abs	TPO Abs	APC Abs	Auto immune disease development	Follow-up (years)	Therapy
1	18	−	0	−	−	+	−	HT	2	Levotiroxina
2	28	+	5	+ (AAI abd GAD Abs)	−	−	−	−	0	–
3	31	+	11	+ (IA-2 Abs)	−	−	−	−	0	–
4	34	−	0	+ (Znt8 Abs)	−	−	−	−	0	–
5	39	−	0	+ (GAD and IA-2 Abs)	−	+	−	T1DM	4	Insulin
6	43	−	0	+ (GAD Abs)	−	−	−	−	2	–

MAA multi-autoantigen radioimmunoassay, *GAD Abs* glutamic acid decarboxylase, *IA-2 Abs* tyrosine phosphatase autoantibodies, *ZnT8 Abs* islet beta-cell zinc cation efflux transporter autoantibodies, *21-OH Abs* 21-hydroxylase antibodies, *TPO Abs* thyroid peroxidase antibodies, *APC Abs* autoantibodies against the gastric parietal cells

(8/122, 95 % CI 2.2–10.8), respectively. In one case, the thyroid-specific autoimmune marker was combined with diabetes-specific immunoreactivity (Table 2).

Gastric-specific autoimmunity

None of the 61 KS patients was positive for gastric parietal cell autoantibodies. One of the 122 controls (0.8 %, 1/122, 95 % CI −0.8 to 2.4) showed gastric-specific autoimmunity.

Endocrine organ-specific humoral autoimmunity in 47,XXY KS patients subdivided by TRT

The autoantibody frequency distribution in KS patients with no history of previous TRT (Group 1), in KS patients undergoing TRT (Group 2), and in controls is reported in Fig. 2.

Serum samples from 4 (11.4, 95 % CI 0.9–21.9) of the 35 TRT-naïve KS patients were positive for at least one of the organ-specific autoantibodies investigated. This frequency was not significantly higher than the 7.7 % (2/26, 95 % CI −2.5 to 17.9) found in samples from the 26 KS patients undergoing TRT.

Comparison of the distribution of organ-specific autoantibody frequency in the two KS groups revealed that diabetes-specific autoimmunity was 8.6 % (3/35, 95 % CI −0.7 to 17.9) in TRT-naïve and 7.7 % (2/26, 95 % CI −2.5 to 17.9) in TRT-treated KS patients. Furthermore, it was significantly higher in TRT-naive patients than in controls (3/35 vs. 1/122, $p = 0.034$). The difference between Group 1 and 2 KS patients was not significant, as well as between Group 2 KS patients and controls. TPO Abs positivity was found only in Group 1 (5.7 %, 2/35, 95 % CI −1.7 to 13.1) with a frequency similar to that observed in the control

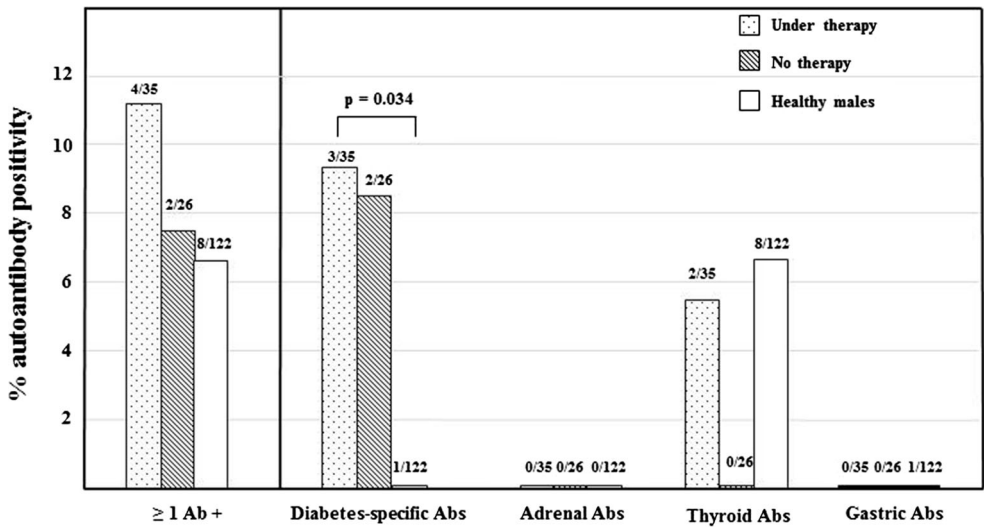

Fig. 2 Frequency of endocrine autoantibody specificities in 47,XXY KS patients with no history of previous hormone replacement therapy (Group 1), in patients undergoing therapy (Group 2) and in healthy men. Diabetes-Specific Abs: glutamic acid decarboxylase (GAD Abs), insulin (Insulin Abs), tyrosine phosphatase (IA-2 Abs), and islet beta-cell zinc cation efflux transporter (ZnT8 Abs) autoantibodies; Adrenal Abs: 21-hydroxylase antibodies; Thyroid Abs: thyroid peroxidase antibodies (TPO Abs); Gastric Abs: autoantibodies to the gastric parietal cell (APC Abs)

group (6.5 %, 8/122). Thyroid-specific immunoreactivity of KS patients undergoing TRT was not significantly lower than in the control group (0/26 vs. 8/122). No KS patient or control was positive for 21-OH Abs or APC Abs and only one control was found positive for gastric-specific autoimmunity.

Autoantibody-positive 47,XXY KS patients

The clinical and autoimmune patterns of the autoantibody-positive KS patients (9.8 %, 6/61) are presented in Table 2. Four of these six patients had no history of TRT, while two were undergoing TRT at the time of blood sample collection (mean therapy duration: 8 years, range 5–11 years).

The serum sample from one KS patient was positive for different organ-specific immunoreactivities, thus placing him at risk of developing polyendocrinopathy. In this case, diabetes-specific autoimmunity (GAD and IA-2) was associated with a positivity for TPO Abs. In the other five patients, only single organ-specific immunoreactivities were detected, with one serum sample positive for anti-TPO Abs and the other four positive for diabetes-specific Abs (GAD Abs, Znt8 Abs, GAD/Insulin Abs, and IA-2, respectively).

One autoantibody-positive patient from Group 1 (1/4) started TRT during the study. Comparison of endocrine organ-specific humoral immunoreactivity before and after the beginning of TRT revealed a decrease in humoral response (data not shown). The two immunoreactive KS patients in Group 2 began TRT before being enrolled in the study, thus preventing comparison of autoantibody titers before and after the beginning of TRT.

Discussion

This study investigated for the first time the endocrine autoantibody profile of four different organ-specific autoimmune diseases (T1DM, AD, HT, and autoimmune chronic AG) in the same cohort of 61 adult 47,XXY Klinefelter patients. It is well known that autoimmune diseases generally show sexual dimorphism, predominantly affecting females [5]. For this reason, before analyzing our patients for the presence of the various endocrine autoantibodies, and with the aim of avoiding gender bias in our results, we decided to calculate the limit of positivity for each assay by using an adult cohort of 158 male subjects. For the same purpose, we compared the endocrine autoimmunity profile of our KS patients against that found in double the number of healthy men.

Altogether, we found that 9.8 % of 47,XXY KS patients showed organ-specific endocrine humoral immunoreactivity, with a higher frequency than in the control group (6.5 %).

Literature evidence shows that diabetes mellitus is frequently associated with KS [26, 27]. However, these studies rarely distinguished between type 1 and type 2 diabetes. Clinical information suggests that most patients had T2DM [3]. In 2012, a study analyzing coexisting conditions in T1DM patients reported that 1.9 % of T1DM

patients had KS [28]. It is now well known that the presence of diabetes-specific autoimmunity may help to differentiate type 1 from T2DM [29]. There is only sporadic information, coming essentially from case reports, on the frequency of diabetes-specific immunoreactivity in KS patients [30, 31]. This is essentially due to the low number of autoantibodies (in particular diabetes-specific) identified at the time of these studies and possibly also to the low sensitivity and specificity of the available assays. In this study, we evaluated diabetes-specific humoral immunoreactivities by using an innovative assay that achieved high sensitivity and specificity at the 2010 Diabetes Antibody Standardization Program [16]. We found that 8.2 % of 47,XXY KS patients were positive for at least one of the four diabetes-specific autoantibodies investigated, with no significant difference between KS patients receiving or not receiving TRT. Interestingly, irrespective of TRT, the diabetes-specific autoantibody frequency in KS was significantly higher than in the healthy controls used in our study and was comparable to that described in literature for first degree relatives of T1DM patients [32, 33]. As reported in the literature, the presence of two or more of these autoantibodies confers a >50 % risk of developing T1DM within 5 years in relatives of patients with T1DM [34, 35], and GAD65 and IA-2 antibodies are the strongest predictors of T1DM [36].

In our cohort of KS immunoreactive patients, two serum samples showed double positivity for diabetes-specific immunoreactivities, with GAD and IA-2 autoantibodies more frequent than the other diabetes-specific autoantibodies. It is worth noting that the GAD- and IA-2 autoantibody-positive KS patient in this cohort went on to develop clinical T1DM and is currently under insulin treatment. The other case, namely the GAD- and Insulin-Abs positive KS patient, shows no clinical or biochemical evidence of autoimmune diabetes to date.

In line with the already known higher susceptibility of T1DM patients to other autoimmune disorders [37], the KS patient with T1DM was also positive for anti-TPO autoantibodies, the marker of Hashimoto's thyroiditis. Thyroid autoimmunity was present in 3.3 % of KS patients, with no significant differences compared to the control group. The TPO antibody frequency in TRT-naïve patients (5.7 %) was similar to that found in previous studies (5.4 %) [8, 9] and was higher in these patients than in those undergoing TRT.

To our knowledge, this is the first study investigating gastric parietal and adrenal humoral autoimmunity in KS. These endocrine immunoreactivities were not found in our cohort of 47,XXY KS patients.

In conclusion, our results provide additional weight to the already known concept of enhanced immune response in KS patients and, for the first time, we report a comprehensive pattern of endocrine organ-specific autoimmunity in adult Caucasian 47,XXY KS patients. Diabetes-specific humoral immunoreactivity is the most frequent endocrine organ-specific autoimmunity in these patients and is significantly increased in comparison with healthy men.

Autoantibody formation precedes the possible development and progression of most autoimmune disorders. Over the course of our study, these autoantibodies showed clinical significance; the GAD/IA-2 Ab positive patient was diagnosed with T1DM and the TPO Ab positive patient with thyroiditis. Both these patients were asymptomatic at the time of autoantibody detection. They are now being treated respectively with insulin and with levotiroxina (Table 2). The other four KS immunoreactive KS patients were all asymptomatic.

Detection of serum autoantibodies may therefore help in identifying KS patients at risk of developing organ-specific autoimmune diseases (thus preventing morbidity related to unrecognized diseases) and in monitoring the preclinical and clinical phases of the disease. We suggest that screening for diabetes and thyroid-specific autoimmunity in every KS patient and follow-up of the immune response should be considered in KS clinical practice.

Testosterone replacement therapy autoantibody frequencies in TRT-naïve KS patients were comparable to those in patients undergoing TRT. Furthermore, one autoantibody-positive KS patient with low testosterone levels underwent TRT during the study, showing decreasing immunoreactivity (data not shown). As hypothesized by Kocar et al. [38], this reduction might be related at least in part to the immunosuppressive effects of testosterone. However, it is not possible to affirm that TRT does not influence autoimmunity in KS patients, because it is unclear if it might induce large biochemical differences in our treated KS group. Additional longitudinal studies are needed to understand the potential effect of TRT and its duration on autoantibody production in KS. Nevertheless, it is important to underline that not only endocrine but also several other intrinsic and extrinsic etiologic factors such as genetic predisposition and environmental factors may contribute to autoimmune disease progression and pathogenesis. Moreover, the long term consequences of hypergonadotropic hypogonadism in KS patients are difficult to separate from the gene-dose effects of the X chromosome aneuploidy. In addition to the existing literature reports [4, 39, 40], further studies on KS patients with higher X chromosome aneuploidies are needed to clarify the relationship between the gene-dose effect and androgen levels in the development and maintenance of endocrine organ-specific humoral autoimmunity.

Acknowledgments The study was funded by the Italian Ministry of Health and the Italian Medicines Agency (AIFA): research project

MRAR08Q009 on rare diseases. Author's contribution: F.P. contributed to data collection, statistical analysis, data interpretation, wrote the manuscript; C.T. contributed to data interpretation and revised the manuscript; G.S., A.A., and A.S. contributed to data collection; L.A. and G.D. revised the manuscript; R.A. is the guarantor of this work and, as such, had full access to all data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Thanks to Marie-Hélène Hayles for the language revision.

Conflict of interest No potential conflicts of interest relevant to this article were reported.

References

1. F. Lanfranco, A. Kamischke, M. Zitzmann et al., Klinefelter's syndrome. Lancet **364**, 273–283 (2004)
2. A.F. Radicioni, E. De Marco, D. Gianfrilli et al., Strategies and advantages of early diagnosis in Klinefelter's syndrome. Mol. Hum. Reprod. **6**, 434–440 (2010)
3. A. Bojesen, S. Juul, N.H. Bikebaek et al., Morbidity in Klinefelter syndrome: a Danish register study based on hospital discharge diagnoses. J. Clin. Endocrinol. Metab. **88**, 622–626 (2006)
4. A.H. Sawalha, J.B. Harley, R. Hal Scofield, Autoimmunity and Klinefelter's syndrome: when men have two X chromosomes. J. Autoimmun. **33**(1), 31–34 (2009)
5. L.A. Abramowitz, S. Olivier-Van Stichelen, J.A. Hanover, Chromosome imbalance as a driver of sex disparity in disease. J. Genomics **2**, 77–88 (2014)
6. M. Cutolo, A. Sulli, S. Capellino et al., Sex hormones influence on the immune system: basic and clinical aspects in autoimmunity. Lupus **13**(9), 653–658 (2004)
7. N.J. Olsen, W.J. Kovacs, Gonadal steroids and immunity. Endocr. Rev. **17**, 369–384 (1996)
8. C. Oktenli, Z. Yesilova, I.H. Kocar et al., Study of autoimmunity in Klinefelter's syndrome and idiopathic hypogonadotropic hypogonadism. J. Clin. Immuno. **22**, 137–143 (2002)
9. A.M.B. Bjørn, A. Bojesen, C.H. Gravholt, P. Laurberg, Hypothyroidism secondary to hypothalamic-pituitary dysfunction may be part of the phenotype in klinefelter syndrome: a case-control study. J. Clin. Endocrinol. Metab. **94**(7), 2478–2481 (2006)
10. G.S. Eisenbarth, Update in type 1 diabetes. J. Clin. Endocrinol. Metab. **92**, 2403–2417 (2007)
11. M. Knip, H. Siljander, Autoimmune mechanisms in type 1 diabetes. Autoimmun. Rev. **7**, 550–557 (2008)
12. O. Winqvist, F.A. Karlsson, O. Kämpe, 21-Hydroxylase, a major autoantigen in idiopathic Addison's disease. Lancet **339**(8809), 1559–1562 (1992)
13. J. Bednarek, J. Furmaniak, N. Wedlock et al., Steroid 21-hydroxylase is a major autoantigen involved in adult onset autoimmune Addison's disease. FEBS Lett. **309**(1), 51–55 (1992)
14. A.P. Weetman, Chronic autoimmune thyroiditis, in *Werner and Ingbar's the thyroid: a fundamental and clinical text*, 8th edn., ed. by L.E. Braverman, R.D. Utiger (Lippincott Williams & Wilkins, Philadelphia, 2000), pp. 721–732
15. R.G. Strickland, I. Mackay, A reappraisal of the nature and significance of chronic atrophic gastritis. Am. J. Dig. Dis. **18**, 426–440 (1973)
16. C. Tiberti, L. Yu, F. Lucantoni et al., Detection of four diabetes specific autoantibodies in a single radioimmunoassay: an innovative high-throughput approach for autoimmune diabetes screening. Clin. Exp. Immunol. **166**(3), 317–324 (2011)
17. P. Vardi, S.A. Dib, M. Tuttleman et al., Competitive insulin autoantibody RIA: prospective evaluation of subjects at high risk for development of Type 1 diabetes mellitus. Diabetes **36**, 1286–1291 (1987)
18. C.E. Grubin, T. Daniels, B. Toivola et al., A novel radioligand binding assay to determine diagnostic accuracy of isoform-specific glutamic acid decarboxylase antibodies in childhood IDDM. Diabetologia **37**, 344–350 (1994)
19. C. Törn, P.W. Mueller, M. Schlosser et al., Diabetes antibody standardization program: evaluation of assays for autoantibodies to glutamic acid decarboxylase and islet antigen-2. Diabetologia **51**, 846–852 (2008)
20. C. Tiberti, A. Verrienti, B. Fiore et al., IA-2 combined epitope assay: a new, highly sensitive approach to evaluate IA-2 humoral autoimmunity in type 1 diabetes. Clin. Immunol. **115**, 260–267 (2005)
21. J.M. Wenzlau, K. Juhl, L. Yu et al., The cation efflux transporter ZnT8 (Slc30A8) is a major autoantigen in human type 1 diabetes. Proc. Natl. Acad. Sci. USA. **104**, 17040–17045 (2007)
22. V. Lampasona, M. Schlosser, P.W. Mueller, A.J.K. Williams, J.M. Wenzlau, J.C. Hutton, P. Achenbach, Participating Laboratories, Diabetes antibody standardization program: first proficiency evaluation of assays for autoantibodies to zinc transporter 8. Clin. Chem. **57**(12), 1693–1702 (2011)
23. A. Falorni, S. Chen, R. Zanchetta et al., Measuring adrenal autoantibody response: Interlaboratory concordance in the first international serum exchange for the determination of 21-hydroxylase autoantibodies. Clin. Immunol. **140**, 291–299 (2011)
24. G. Huber, J.J. Staub, C. Meier et al., Prospective study of the spontaneous course of subclinical hypothyroidism: prognostic value of thyrotropin, thyroid reserve, and thyroid antibodies. J. Clin. Endocrinol. Metab. **87**, 3221–3226 (2002)
25. S. Whittingham, I.R. Mackay, Pernicious anemia and gastric atrophy, in *The Autoimmune Diseases*, ed. by N.R. Rose, I.R. Mackay (Academic Press, New York, 1985), pp. 243–266
26. M. Jang-Feng, X. Hong-Li, W. Xue—Yan et al., Prevalence and risk factors of diabetes in patients with Klinefelter syndrome: a longitudinal study. Reprod. Endocrinol. **98**, 1331–1335 (2012)
27. A. Bojesen, K. Kristensen, N.H. Birkebaek, J. Fedder, L. Mosekilde, P. Bennett, P. Laurberg, J. Frystyk, A. Flyvbjerg, J.S. Christiansen, C.H. Gravolt, The metabolic syndrome Is frequent in Klinefelter's syndrome and is associated with abdominal obesity and hypogonadism. Diabetes Care **29**, 1591–1598 (2006)
28. S. Kota, K.M. Lalit, S. Jammula, S.K. Kota, D.M. Kirtikumar, Clinical profile of coexisting conditions in type 1 diabetes mellitus patients. Diabetes Metab. Syndr. **6**, 70–76 (2012)
29. A.L. Notkins, A. Lernmark, Autoimmune type 1 diabetes: resolved and unresolved issues. J. Clin. Invest. **108**(12), 47–52 (2001)
30. X.P. Cai, L. Zhao, M. Mao, A case of Klinefelter's syndrome with type 1 diabetes mellitus. Chin. Med. J. **125**(5), 937–940 (2012)
31. K. Ota, T. Suehiro, Y. Ikeda, K. ArII, Y. Kumon, K. Hashimoto, Diabetes mellitus associated with Klinefelter's syndrome: a case report and review in Japan. Intern. Med. **41**(10), 842–847 (2002)
32. L.I. Alves, E. Davini, M.R. Correia, R.T. Fukui, R.F. Santos, M.R. Cunha, D.M. Rocha, W.M.G. Volpini, M.E.R. Silva, Autoantibodies and high-risk HLA susceptibility markers in first-degree relatives of Brazilian patients with type 1 diabetes mellitus: a progression to disease based study. J. Clin. Immunol. **32**, 778–785 (2012)
33. C. Verge, E. Gianani, L. Kawasaki et al., Prediction of type I diabetes in first-degree relatives using a combination of insulin, GAD, and ICA512bdc/IA-2 autoantibodies. Diabetes **45**, 926–993 (1996)
34. N.K. Mclaren, M.S. Lan, D. Schatz et al., Multiple autoantibodies as predictors of type 1 diabetes in a general population. Diabetologia **46**(6), 873–874 (2003)
35. J.M. Barker, J. Yu, L. Yu et al., Autoantibody "subspecificity" in type 1 diabetes: risk for organ-specific autoimmunity clusters in distinct groups. Diabetes Care **28**, 850–855 (2005)

36. M. Pietropaolo, R. Towns, G.S. Eisenbarth, Humoral autoimmunity in type 1 diabetes: prediction, significance, and detection of distinct disease subtypes. Cold Spring Harb. Perspect. Med. **2**(10), 1–18 (2012)
37. O. Kordonouri, D. Deiss, T. Danne et al., Predictivity of thyroid autoantibodies for the development of thyroid disorders in children and adolescents with Type 1 diabetes. Diabet. Med. **19**(6), 518–521 (2002)
38. I.H. Kocar, Z. Yesilova, M. Ozata et al., The effect of testosterone replacement treatment on immunological features of patients with Klinefelter's syndrome. Clin. Exp. Immunol. **121**, 448–452 (2000)
39. J. Nielsen, K. Johansen, H. Yde, Frequency of diabetes mellitus in patients with Klinefelter's syndrome of different chromosome constitutions and the XYY syndrome. Plasma insulin and growth hormone level after a glucose load. J. Clin. Endocrinol. Metab. **29**, 1062–1073 (1969)
40. J. Visootsak, J.M. Graham Jr, Klinefelter syndrome and other sex chromosomal aneuploidies. Orphanet J. Rare Dis. **1**, 42 (2006)