SOX13 gene downregulation in peripheral blood mononuclear cells of patients with Klinefelter syndrome

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Klinefelter syndrome (KS) is the most common sex chromosome disorder in men. It is characterized by germ cell loss and other variable clinical features, including autoimmunity. The sex-determining region of Y (SRY)-box 13 (SOX13) gene is expressed in mouse spermatogonia. In addition, it has been identified as islet cell autoantigen 12 (ICA12), which is involved in the pathogenesis of autoimmune diseases, including type 1 diabetes mellitus (DM) and primary biliary cirrhosis. SOX13 expression has never been investigated in patients with KS. In this age-matched, case–control study performed on ten patients with KS and ten controls, we found that SOX13 is significantly downregulated in peripheral blood mononuclear cells of patients with KS compared to controls. This finding might be consistent with the germ cell loss typical of patients with KS. However, the role of SOX13 in the pathogenesis of germ cell loss and humoral autoimmunity in patients with KS deserves to be further explored.

Asian Journal of Andrology (2021) 23, 157–162; doi: 10.4103/aja.aja_37_20; published online: 27 October 2020

Keywords: germ cells; intellectual disability; Klinefelter syndrome; rare disease; sex-determining region of Y-box 13 (SOX13)

INTRODUCTION

Klinefelter syndrome (KS) is the most common sex chromosome disorder in men, with an estimated prevalence of 1:500 to 1:1000 newborns.¹ The most widespread karyotype in men with KS is 47,XXY (the so-called “classic” nonmosaic karyotype). Classic nonmosaic karyotype occurs in approximately 80%–90% of men with KS² and is due to paternal meiotic nondisjunction in 50% of cases.¹ Otherwise, mosaic KS (e.g., 47,XXY/46,XY) and other nonmosaic forms, such as complex karyotype or other numeric sex chromosome abnormalities (e.g., 48, XXXY, 48, XXXY, and 49, XXXXY), can be found in the remaining patients.³,⁴

The abnormal karyotype leads to progressive germ cell degeneration starting from mid-puberty, impaired Sertoli cell (SC) function,⁵ total tubular atrophy or hyalinizing fibrosis, and relative hyperplasia of Leydig cells.⁶ Occasionally, foci of spermatogenesis have been observed in the testes of men with KS.⁶ Clinically, azoospermia occurs in the majority of patients with nonmosaic KS. In addition, sperm has been found in 7.7%–8.4% of patients with (apparently) nonmosaic KS.²

Several other clinical manifestations can be associated with the syndrome, such as learning and developmental disability, personality disorder and behavioral problems, intelligence quotient (IQ) lower by 10–15 points but not in the intellectual disability range, increased risk for mitral valve prolapse, lower-extremity varicose veins, venous stasis ulcers, deep-vein thrombosis, pulmonary embolism, autoimmune diseases, 20-fold-higher risk of developing breast cancer, type 2 diabetes mellitus (T2DM), metabolic syndrome, extragonadal germ cell tumors, and non-Hodgkin lymphoma.¹⁵–⁹

Despite an increasing number of studies investigating the gene expression profile in both peripheral blood mononuclear cells (PBMCs) and, when available, in the testicular tissue of patients with KS,¹⁰–¹⁰ the molecular mechanisms responsible for germ cell degeneration remain elusive. It has been hypothesized that the escape of inactivation of genes on the supernumerary X chromosome might affect germ cell development and/or meiosis.²² However, transcriptome analysis of testicular tissue of men with KS resulted in the normal expression of X-linked genes.²⁰ By contrast, deregulation of gene mapping on autosomes has been shown in men with KS and, therefore, the supernumerary X chromosome has been suggested to influence the regulation of these genes.¹⁴

The sex-determining region of Y (SRY)-box 13 (SOX13) maps to the 1q32.1 chromosome. It belongs to the family of SRY-related high-mobility group (HMG)-BOX genes, which, in turn, encode a group of transcription factors with an HMG-type DNA-binding domain. The latter consists of three α-helices whose binding to specific DNA sequences influences DNA structure and transcription.²⁵,²³ In mice, members of the Sox transcription factor family play a role in fetal development in multiple tissues, including the testis.²³ Accordingly, SRY, required for male sex determination in both humans and mice, targets the sex-determining region of Y-box 9 (Soxx9) expression, which initiates Sertoli cell differentiation.²⁵,²⁶
Recently, SOX13 has been found to be expressed in mouse type A and B spermatogonia. Interestingly, SOX13 is also a diabetes autoantigen expressed in pancreatic cells. No data are currently available on its expression in men with KS. Therefore, this study was undertaken to evaluate whether differential SOX13 gene expression occurs in peripheral blood mononuclear cells (PBMCs) of men with KS compared with healthy controls.

**PATIENTS AND METHODS**

**Patients, controls, and RNA extraction**

Ten men with KS with the nonmosaic KS karyotype 47,XXY (as confirmed by cytogenetic investigation performed on at least fifty metaphases) and ten healthy age-matched controls with 46,XY karyotype, no clinical history of genetic diseases, normal testicular volume, and normal reproductive hormone (gonadotropins and total testosterone [TT]) levels were recruited. Patients and controls were Italians. They were evaluated for gonadotropins, TT levels, body mass index (BMI), glycemia, and serum insulin levels. Insulin resistance was calculated using the homeostasis model assessment index-insulin resistance (HOMA-IR).

Fitting with the diagnosis, all patients with KS had azoospermia, increased follicle-stimulating hormone (FSH) serum levels, and low testicular volumes. The clinical and biochemical parameters of each man with KS and control have already been reported. Patients and controls were age matched (mean ± standard deviation [s.d.]: 32.4 ± 8.1 vs 33.1 ± 7.9 years, P > 0.1) and did not differ in BMI, glycemia, insulin, or HOMA-IR. As expected, serum gonadotropins and TT levels were significantly different in patients with KS compared to controls (P < 0.05; Table 1). Among patients with KS, five were on testosterone replacement therapy (TRT). No KS or control was diabetic. An increased HOMA index, consistent with insulin resistance, was found in 42.9% (3/7) of men with KS and 20.0% (1/5) of controls (*P* < 0.05, patients versus controls). Interestingly, SOX13 is also a diabetes autoantigen expressed in pancreatic cells. No data are currently available on its expression in men with KS. Therefore, this study was undertaken to evaluate whether differential SOX13 gene expression occurs in peripheral blood mononuclear cells (PBMCs) of men with KS compared with healthy controls.

**Table 1: Clinical and biochemical parameters of men with nonmosaic Klinefelter syndrome and age-matched controls**

| Parameters               | Patients Mean±s.d. | Controls Mean±s.d. |
|--------------------------|--------------------|---------------------|
| Age (year)               | 32.4±8.1           | 33.1±7.9            |
| BMI (kg m⁻²)             | 26.0±6.7           | 25.1±2.7            |
| Glycerina (mg dl⁻¹)      | 81.2±14.7          | 87.8±8.3            |
| Insulin (µU ml⁻¹)        | 29.7±44.1          | 15.2±13.7           |
| HOMA-IR                  | 6.2±9.3            | 3.6±3.5             |
| LH (IU l⁻¹)              | 20.9±7.6           | 5.0±2.1             |
| FSH (IU l⁻¹)             | 32.7±16.9*         | 3.5±0.6             |
| TT (ng ml⁻¹)             | 3.8±2.4*           | 5.9±1.8             |
| Total sperm count (10⁶ per ejaculate) | 270.6±132.6        |

Age: BMI, LH, FSH, TT, and testicular volume values for each patient and control are detailed in the study of Cinino et al. Normal ranges of glycemia: 60–100 mg dl⁻¹; insulin: 1.9–23 µU ml⁻¹; LH: 1.14–8.75 IU l⁻¹; FSH: 0.95–11.95 IU l⁻¹; TT: 2.5–9.8 ng ml⁻¹. *P*<0.05, patients versus controls (Student’s *t*-test). s.d.: standard deviation; BMI: body mass index; HOMA-IR: homeostasis model assessment of insulin resistance; LH: luteinizing hormone; FSH: follicle-stimulating hormone; TT: total testosterone.

**RNA sequencing and data analysis**

Indexed libraries were prepared from 1 μg of purified RNA with the TruSeq Stranded Total RNA (Illumina, Eindhoven, The Netherlands) Library Prep Kit according to the manufacturer’s instructions. The libraries were quantified using the Agilent 2100 Bioanalyzer (Agilent Technologies) and pooled such that each index-tagged sample was present in equimolar amounts, with a final concentration of the pooled samples of 2 nmol l⁻¹. The pooled samples were subjected to cluster generation and sequencing using an Illumina HiSeq 2500 System (Illumina) in a 2 x 100 paired-end format. The raw sequence files generated (.fastq files) underwent quality control analysis using FastQC (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/).

Bioinformatics analysis was performed by Genomix4Life Srl (”Schola Medica Salernitana,” Baronissi, SA, Italy). The quality-checked reads were trimmed with cutadapt v.1.10 (https://cutadapt.readthedocs.io/en/v1.10/changes.html#v1-10) and then aligned to the human genome (hg19 assembly) using STAR v.2.5.2a with standard parameters. Differentially expressed mRNAs were identified using DESeq2 v.1.12.

Gene annotation, as provided by Ensembl (GRCh37; https://grch37.ensembl.org/index.html), was obtained for all known genes in the human genome. We calculated the number of reads mapping to each transcript with HTSeq-count v.0.6.1. These raw read counts were then used as input to DESeq2 for calculation of normalized signal for each transcript in the sample, and differential expression was reported as the fold change along with the associated adjusted P values (computed according to Benjamini–Hochberg). Differential expression data were further confirmed using Cuffdiff36.

**Validation with real-time quantitative reverse transcription-polymerase chain reaction (qRT-PCR)**

To validate the results obtained by NGS analysis, we compared the RT-PCR results from ten patients with KS and ten normal controls. qRT-PCR was performed as described elsewhere. cDNA transcription was carried out for each sample using a cDNA synthesis kit (Thermo Scientific Maxima First Strand cDNA Synthesis Kit for RT-qPCR) according to the manufacturer’s instructions. Real-time PCR analysis for SOX13 was performed using TaqMan Gene Expression Assay primers. Briefly, total RNA was extracted from samples using TRIzol reagent (Sigma-Aldrich, Milan, Italy) and quantified by reading the optical density at 260 nm. In particular, 2.5 µg of total RNA was subjected to reverse transcription (RT, Thermo Scientific) in a final volume of 20 µl. qPCR was performed using 25 ng of cDNA prepared by RT and SYBR Green Master Mix (Stratagene, Agilent Technology, Amsterdam, The Netherlands). This was performed in an Mx3000P cycler (Stratagene), using FAM for detection and ROX as the reference dye. The mRNA level of each sample was normalized against glyceraldehyde-3-phosphate dehydrogenase.
Figure 1: NGS analysis. (a) Screenshot from Integrative Genomics Viewer (IGV) for SOX13. Three control samples and three Klinefelter samples among the ten samples are displayed. (b) Histograms showing the expression (FPKM) in the twenty sequenced samples. The control samples are shown in red, whereas the Klinefelter samples are shown in blue. (c) Boxplot showing the SOX13 expression (FPKM) in control samples (in red) and Klinefelter samples (in blue). SOX13 expression is lower in Klinefelter samples. \( P < 0.05 \). NGS: next-generation sequencing; FPKM: fragments per kilobase of transcript per million; SOX13: sex-determining region of Y-box 13.

RESULTS

Integrative Genomics Viewer for SOX13 in three KS patients and three controls (Figure 1a), revealed a quantitatively reduced expression of SOX3 in patients than controls, as confirmed by the analysis of expression of the twenty consecutive samples (Figure 1b). Overall, NGS transcriptome analysis revealed that the SOX13 gene (locus 1:204042424-204096863) was downregulated in patients with KS by \(-3.701\)-fold \((Q < 0.05)\) compared with controls (Figure 1c). The raw data of this research project are available in the ArrayExpress database repository (https://www.ebi.ac.uk/arrayexpress/) with accession number E-MTAB-6107.

In our case–control study with qRT-PCR, we used all KS cases and controls and, specifically, we obtained a mean FC of cases of 0.48 (s.d.: 0.25; Figure 2). Statistical analysis revealed a significant difference between the control and KS groups \((P < 0.05)\). The mean of KS cases was obtained with the Software Version 1.5 supplied with the LightCycler\textsuperscript{®} 480, as previously reported.\textsuperscript{31}

Distribution analysis of measured gene transcript levels was performed using the Shapiro–Wilk test, and statistical analysis of the results was carried out using paired two-tailed \(t\)-test and bivariate linear regression analysis. GraphPad Prism 5 software (https://www.graphpad.com/scientific-software/prism/) was used for statistical analysis. \( P < 0.05 \) was accepted as statistically significant.

Differential expression data were further confirmed using Cuffdiff36. Raw data are available in the ArrayExpress database repository (https://www.ebi.ac.uk/arrayexpress/) with accession number E-MTAB-6107.

DISCUSSION

Impaired spermatogenesis with total tubular atrophy or hyalinizing fibrosis is the most common histological testicular feature of men with KS.\textsuperscript{1} Although a number of transcriptome studies have been performed both in PBMCs and in testicular tissue from patients with KS, the molecular mechanism responsible for germ cell degeneration in KS is not yet understood. Its acknowledgment would be of great interest to address future target gene therapies.

In the present study, we report, for the first time, that the SOX13 gene is downregulated in PBMCs from patients with KS compared to controls. The SOX13 gene belongs to the SOX family, whose members are involved in testicular differentiation in most vertebrates. In mice, the Sox gene family encodes a group of transcription factors with an HMG-box DNA-binding domain that is similar to that of the sex-determining region of the Y (Sry) protein. Sox genes are classified into eight groups, named from A to H. In particular, the Sox13 group includes Sox5, Sox6, and Sox13 in most vertebrates.\textsuperscript{32} Sox proteins are known to be involved in testicular differentiation. In particular, Sox9 is tightly associated with SC differentiation\textsuperscript{33–35} and might also influence testosterone production by Leydig cells. Furthermore, Sox4, Sox11, and Sox12 protein expression has been found in the mouse testis during development,\textsuperscript{36} while that of Sox9, Sox5, and Sox13 has been found in the seminiferous tubules of the postnatal mouse testis.\textsuperscript{37} Sox proteins are likely involved in spermatogenesis. Accordingly, Sox4, Sox8, Sox9, and Sox12 proteins are highly expressed in SCs and Sox5, Sox6, and Sox30 in spermatocytes and spermatids, whereas Sox3, Sox4, Sox12, and Sox13 have been detected in spermatogonia of both mice and rats.\textsuperscript{37}

The role that Sox proteins display in spermatogenesis has been proven by knockout studies. Accordingly, Sox30 knockout mice show infertility due to arrested spermatogenesis at the spermatid phase. This protein seems to address haploid gene transcription in the late meiosis and spermigenesis phases. In contrast, this role has not been observed in mouse female gametogenesis.\textsuperscript{38–40} In addition, Sox4, which is known to be involved in gonadal morphogenesis, is involved in germ cell differentiation in male mice. Indeed, Sox4 deficiency results in the reduction in mouse germ cell differentiation markers, such as Nanos\textsubscript{2} and DNA methyltransferase 2-like protein (Dnmt3l), and increased pluripotency gene expression. Instead, female germ cells normally enter meiosis.\textsuperscript{41}
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SoxD proteins have two conserved functional domains: the family-specific HMG box DNA-binding domain in the C-terminal part and the group-specific coiled coil in the N-terminal region of the protein. In mouse proteins, these domains share 87% and 76% homology with the N-terminal and C-terminal domains of the human SOXD proteins, respectively. This likely supports that Sox proteins may have a conserved function among vertebrates, including humans, where their role in spermatogenesis cannot be excluded.

SoxD proteins are involved in transcriptional activation and repression. In particular, Sox13 has been shown to modulate canonical Wingless-type MMTV integration site family (Wnt) signaling. Interestingly, FSH enhances type A undifferentiated spermatogonia (Aund) proliferation via Leydig cell-derived Wingless-type MMTV integration site family, member 5a (Wnt5a) production. Accordingly, an in vitro study reported that Wnt5a maintains the number of murine spermatogonial stem cells (SSCs) by activating the c-Jun N-terminal kinase (JNK) pathway. In addition, FSH-induced secretion of insulin-like growth factor 3 (Igf3) in Sertoli cells from zebrafish induces Aund differentiation into type A spermatogonia (Aad) via β-catenin, which is a component of Wnt signaling. Therefore, by modulating the Wnt signaling pathway, Sox13 might be involved in the maintenance of the SSC number and in Aad differentiation.

Overall, these data point to a role for Sox13 in mouse and rat spermatogenesis. The homology of Sox domains with the human SOX proteins indicates that, being highly conserved in vertebrates, they might likely display similar functions among species. Furthermore, the strong similarity between mice and human spermatogenesis has led to a rapid increase in the list of genes recently discovered to be involved in human spermatogenetic failure, mainly based on mouse and rat studies. Although no data have been provided on the role of the SOX13 gene in human spermatogenesis, it may likely be involved in human spermatogenesis due to its expression in mouse and rat spermatogonia.

We found SOX13 downregulation in PBMCs from patients with KS. Some studies have recently addressed a diagnostic role of NGS analysis in PBMCs of patients with apparently idiopathic nonobstructive azoosperma because the mutation of genes involved in spermatogenesis can be detected in the blood. It cannot be excluded that SOX13 downregulation found in PBMCs may also occur in KS germ cells, leading to their apoptosis. Recent research has highlighted the role of SOX13 in cell proliferation. In greater detail, it has been found to enhance paired box gene 8 (PAX8) protein expression, in turn promoting the proliferation of gastric carcinoma cells. In addition, SOX13 upregulates angiogenesis in gliomas. Taken together, these findings may suggest a role for SOX13 in cell proliferation. In view of its expression at the spermatogonial level, SOX13 may also be involved in germ cell proliferation.

The evidence suggests a role for SOX13 dysregulation in the development of autoimmune diseases. By modulating the Wnt signaling pathway, Sox13 protein is involved in the emergence of gamma-delta T-cells in the thymus, opposing alpha-beta T cell differentiation, as the analysis of fetuses with Sox13 gene gain-of-function and loss-of-function mutations suggests. Accordingly, SOX13 has been identified as islet cell autoantigen 12 (ICA12), which is involved in the pathogenesis of autoimmune diseases, including type 1 DM and primary biliary cirrhosis. It is noteworthy that endocrine organ-specific humoral autoimmunity is not rare in patients with KS. Data from 61 patients with KS and 122 controls indicate that it is more frequently directed against type 1 diabetes-related autoantigens (insulin, glutamate decarboxylase [GAD], islet antigen 2 [IA-2], and zinc transporter 8 [Znt8] antibodies), although the prevalence of type 1 DM is low in these patients (few cases have been reported so far). Therefore, the possible role of SOX13 downregulation in the pathogenesis of autoimmune disorders in patients with KS deserves to be examined.

Our results must be taken with care because no data from testicular tissue was available in the present study. Accordingly, none of the patients gave their consent to proceed with testicular biopsy. We are aware that this presents a limit for understanding the role of SOX13 in spermatogenesis. However, the vast majority of transcriptome studies on KS patients have analyzed the transcriptome from the blood due to the limitation in having testicular tissue. This is particularly true nowadays when the testicular biopsy is used to retrieve spermatozoa for assisted reproductive techniques (ARTs). We think that the results of the present study may prompt to develop further focused analysis in centers (or countries) where testicular biopsy of KS patients is readily available.

On the other hand, it could be speculated that the study of SOX13 expression in testicular tissue from adults with KS would not be effective in finding SOX13 downregulation because this tissue already lacks germ cells. Therefore, testicular Sox13 expression would reflect germ cell loss in KS patients. By contrast, blood downregulation might hypothetically reveal a molecular dysfunction possibly occurring in germ cells, prior to and, maybe, favoring, their loss. However, further studies should be performed in aborted fetuses with KS with the aim of assessing Sox13 expression in KS germ cells.

Another reason to take with care our results is that, unfortunately, no data on testicular histology could be provided as patients did not give their consent. Indeed, total testicular volume is low in patients with KS. Testicular fine-needle biopsy would further reduce this volume, thus reducing the success rate of ART in patients willing to undergo this procedure later in life. However, the most typical histologic feature of KS patients is Sertoli cell-only syndrome (SCOS) and, because all the enrolled patients were azoospermic, it could be supposed that they had SCOS.

CONCLUSIONS

The present study reports, for the first time, a downregulation of the SOX13 gene in the PBMCs of patients with KS compared to controls. Data from animal studies indicate a role for Sox13 in SSC maintenance and immune system regulation. Further studies are needed to establish whether SOX13 is involved in the pathogenesis of germ cell loss and endocrine organ-specific humoral autoimmunity in patients with KS.
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AUTHOR CONTRIBUTIONS
RC conceived the study, participated in data analysis, and wrote the original draft. MS conceived the study, participated in genetic analysis, and wrote the original draft. RAC participated in data analysis and project supervision. LC and SLV supervised the project. GG performed the genomic studies and participated in the statistical analysis. GM, AC, and CR participated in the genomic studies and in data analysis. AEC conceived the study, supervised the project, and edited the final version of the manuscript. All authors have read and approved the final version of the manuscript, and agree with the order of presentation of the authors.

COMPETING INTERESTS
All authors declare no competing interests.

REFERENCES
1 Bonomi M, Rochira V, Pasqualli D, Balercia G, Jannini EA, et al. Klinefelter syndrome (KS): genetic, clinical phenotype and hypogonadism. J Endocrinol Invest 2017; 40: 123–34.
2 Maiburg M, Repping S, Giltay J. The genetic origin of Klinefelter syndrome and its effect on spermatogenesis. Fertil Steril 2012; 98: 253–60.
3 Morais da Silva S, Hacker A, Harley V, Goodfellow P, Swan A, et al. SoxExpression during gonadal development implies a conserved role for the gene in testis differentiation in mammals and birds. Nat Genet 1996; 14: 62–8.
4 Vidal VP, Chaboissier MC, de Rooij DG, Schedl A. Sox9 induces testis development in XX transgenic mice. Nat Genet 2001; 28: 216–7.
5 D’Aurora M, Ferlin A, Garolla A, Franchi S, D’Onofrio L, et al. SOX30 is required for male fertility in mice. Dev Biol 2018; 424: 32–9.
6 Kent J, Wheatley SC, Andrews JE, Sinclair AH, Koopman P. A male-specific role for SOX9 in vertebrate sex determination. Development 1996; 122: 2781–22.
7 Schepers GE, Teasdale RD, Koopman P. Twenty pairs of sex: extent, homology, and nomenclature of the mouse and human sex transcription factor gene families. Dev Cell 2002; 3: 167–70.
8 Grosschedl R, Giese K, Pagel J. HMG domain proteins: architectural elements in the assembly of nucleoprotein structures. Trends Genet TIG 1994; 10: 94–100.
9 Daigle M, Roumou P, Martin L. Expressions of Sox9, Sox1, and Sox13 transcription factors in mice tests during postnatal development. Mol Cell Biochem 2015; 407: 209–21.
10 Barrionuevo F, Bagheri-Farn S, Klattig J, Kist R, Taketo MM, et al. Homezygous inactivation of Sox9 causes complete XY sex reversal in mice. Biol Reprod 2006; 74: 195–201.
11 Chaboissier MC, Kobayashi A, Vidal VI, Lützkendorf S, van de Kant HJ, et al. Functional analysis of Sox8 and Sox9 during sex determination in the mouse. Development 2004; 131: 1891–901.
12 Vidal VP, Chaboissier MC, de Rooij DG, Schedl A. Sox9 induces testis development in XX transgenic mice. Nat Genet 2001; 28: 216–7.
13 Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol 2014; 15: 550.
14 Anders S, Pyl PT, Huber W. HTSeq-- A Python framework to work with high-throughput sequencing data. Bioinformatics 2015; 31: 166–9.
15 Liak KV, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Method 2001; 26: 402–8.
16 Lefebvre V. The Sox transcription factors--Sox5, Sox6, and Sox13--are key cell fate modulators. Int J Biochem Cell Biol 2010; 42: 349–32.
17 Kent J, Wheatley SC, Andrews JE, Sinclair AH, Koopman P. A male-specific role for SOX9 in vertebrate sex determination. Development 1996; 122: 2781–22.
18爱德华 J, 汤姆 A, 克劳尔 S, 沃恩 A, et al. SoxExpression during gonadal development implies a conserved role for the gene in testis differentiation in mammals and birds. Nat Genet 1996; 14: 62–8.
19 Vidal VP, Chaboissier MC, de Rooij DG, Schedl A. Sox9 induces testis development in XX transgenic mice. Nat Genet 2001; 28: 216–7.
20爱德华 J, 汤姆 A, 克劳尔 S, 沃恩 A, et al. SoxExpression during gonadal development implies a conserved role for the gene in testis differentiation in mammals and birds. Nat Genet 1996; 14: 62–8.
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. *Endocrine* 2016; 52: 157–64.

52 Sakurai T, Iizuka K, Kato T, Takeda J. Type 1 diabetes mellitus and Klinefelter syndrome. *Intern Med* 2019; 58: 259–62.

53 Cai XP, Zhao L, Mao M, Yang ZJ, Xing XY, et al. A case of Klinefelter’s syndrome with type 1 diabetes mellitus. *Chin Med J (Engl)* 2012; 125: 937–40.

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