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Intratesticular testosterone is increased in men with Klinefelter syndrome and may not be released into the bloodstream owing to altered testicular vascularization – a preliminary report

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SUMMARY

Klinefelter syndrome (KS, 47,XXY) is associated with low serum testosterone (T), long thought to arise from disturbed steroidogenesis in Leydig cells. However, intratesticular testosterone (ITT) concentrations were recently found to be normal in a KS mouse model (41,XX^{Y*}). So far, nothing was known about ITT concentrations in human patients with KS. Therefore, ITT, sex hormone-binding globulin (SHBG) and histological parameters were measured in human testicular biopsies of 11 KS patients, 30 azoospermic patients with Sertoli-cell-only syndrome and nine men with normal spermatogenesis as controls. ITT concentrations showed an overall pronounced excess over intratesticular SHBG in molar terms and were significantly increased in men with KS despite of reduced serum T levels. While the ratio of ITT/serum T was markedly increased in KS, the ITT/LH-ratio was comparable between all groups. After finding significantly increased ITT levels in men with KS, a finding even more striking than in the 41,XX^{Y*} KS mouse model, we set out to find a possible 'vascular' explanation for the lack of T release into the testicular blood stream. In testis biopsies from patients, reliable analysis of the vessels is, however, not possible because of the bias resulting from the dissection technique requiring avoidance of larger blood vessels to prevent bleeding. Consequently, the blood vessel constitution was evaluated in whole testis sections from adult male 41,XX^{Y*} and 40,XY* mice (n = 5, each). Indeed, the blood vessel/testes surface ratio correcting for the smaller testes of XX^{Y*} mice was significantly lower in these mice compared with XY* controls. In conclusion, testicular T production does not seem to be impaired in men with KS. On the contrary, ITT concentrations are increased, but not because of increased SHBG activity. The data from the mouse model let us speculate that a reduced vascular bed might be involved in lower release of T into the bloodstream.

INTRODUCTION

The Klinefelter syndrome (KS), characterized by an additional X chromosome (47,XXY), is the most common chromosomal aberration in males and is typically associated with hypergonadotropic hypogonadism, i.e. low serum testosterone (T) levels in the presence of elevated gonadotropins (Lanfranco *et al.*, 2004; Tüttelmann & Gromoll, 2010). Testicular volume is regularly and severely reduced in KS with histology showing Leydig cell hyperplasia, hyalinization of seminiferous tubules and loss of germ cells often leading to Sertoli-cell-only syndrome (SCOS) (Groth *et al.*, 2013). Although the testicular pathophysiology remains poorly understood, peripheral T deficiency has been long thought to arise from disturbed steroidogenesis in Leydig cells. However, in an experimental KS mouse model [41,XX^{Y*} (Wistuba, 2010)] Leydig cells were found to be hyperplastic and hyperreactive to hCG in vitro, and intratesticular testosterone

(ITT) concentrations were comparable to wild-type control males (Wistuba *et al.*, 2010). Possible explanations for this unexpected finding are that either the steroids produced are bound in the testis by androgen-binding protein (ABP), the murine counterpart of sex hormone-binding globulin (SHBG), or that their release into the circulation is hampered.

So far, nothing was known about ITT concentrations in human patients with KS. Therefore, the first aim of this study was to analyse histological parameters and ITT concentrations in biopsies of KS patients as compared to azoospermic patients with normal karyotype and currently unexplained SCOS as well as control men with normal testis histology (obstructive azoospermia). After finding significantly increased ITT levels in men with KS, a finding even more striking than in the 41,XX^{Y*} KS mouse model, we set out to find a possible 'vascular' explanation for the lack of T release into the testicular blood stream. In fact, Foresta *et al.* (2012) previously found that KS patients present significantly reduced artery diameter in several organs, lending support to the hypothesis of disturbed androgen release into the peripheral circulation. In testis biopsies from patients, reliable analysis of the vessels is, however, not possible because of the limited material available and the bias resulting from the dissection technique requiring avoidance of larger blood vessels to prevent bleeding. Because the blood vessel situation in a biopsy will not be representative of the entire cross section of the testis, we employed our 41,XX^{Y*} mouse model to address the question whether testicular blood vessels are altered in the KS testis, which would provide a possible explanation for reduced serum (= peripheral) T in KS.

MATERIAL AND METHODS

Subjects

We enrolled 11 KS patients, 30 azoospermic patients with histological evidence of complete, bilateral SCOS and nine men undergoing vaso-vasostomy for refertilization after vasectomy with normal spermatogenesis as control group. Testicular sperm extraction (TESE) in the latter group was offered as a safety measure in case refertilization should fail. All testicular biopsies were collected for TESE. KS patients showed a non-mosaic 47,XXY karyotype after analysis of at least 30 metaphases and had not received testosterone substitution prior to biopsy. All SCOS patients had a normal 46,XY karyotype, no Y-chromosomal AZF deletions or any other known cause for spermatogenic failure (e.g. testicular tumour, oncologic therapy). All patients underwent a complete physical examination including ultrasonography of scrotal contents. Testicular volume was calculated using the ellipsoid method and summed as bi-testicular volume. All men provided written informed consent for analysis of their clinical data and use of leftover tissue approved by the Ethics Committee of the State Medical Board and the Medical Faculty of the University of Münster.

Hormone measurements

In patients, a venous blood sample was drawn from the cubital vein between 08:00 and 13:30 h. Serum concentrations of FSH, LH and SHBG were determined by immunofluorometric assays (Autodelfia, Perkin Elmer, Freiburg, Germany) and serum testosterone by a commercial ELISA kit (DRG AURICA ELISA Testosterone Kit; DRG Instruments, Marburg, Germany). Intra- and interassay coefficients of variation were <5 and <10% respectively. Free testosterone was calculated from total testosterone and SHBG concentrations (Vermeulen *et al.*, 1999).

ITT levels in human testicular biopsies were measured by a double-antibody RIA using the same method previously described in mice (Wistuba *et al.*, 2010). Each sample was measured in triplicates with different dilutions. The intraassay coefficient of variation was 6.0%. The total ITT content was calculated based on the testicular volume determined by ultrasound assuming 1 g = 1 mL.

In mice, serum T was measured in duplicate from trunk blood samples obtained at sacrifice as published previously (Wistuba *et al.*, 2010). The intra- and interassay coefficients of variation were 4.3 and 5.6%, respectively, and the detection limit of the assay was 0.69 nmol/L.

Morphometrical analysis of human testicular tissue

Bouin's fixed specimens, embedded in paraffin, were sectioned to 4 μm. After deparaffinization and rehydration, the sections were stained with PAS and haematoxylin, dehydrated and mounted. The sections were surveyed under an upright microscope (Axioskop; Zeiss, Oberkochen, Germany) at two different magnifications (objectives 10× and 63×). Images of the sections were captured with a computer-assisted camera system (Axiovision 3.0; Zeiss). Ten randomly chosen tubules per biopsy were investigated for each parameter. Diameters of the tubules and lamina propria were determined at a magnification of 10×. To achieve comparable results, the measurements were always taken on the axis running the shortest distance through the centre of the tubules. We counted Leydig cells magnified at 40× using a grid ocular at random, representative for the whole slice area. Sertoli cells present in 10 different transversally cut tubules per slice were also counted.

Animals

Adult (> 3 months pp) male 41,XX^{Y*} and 40,XY* (n = 5, each) male littermate controls of the strain B6Ei.Lt-Y*/EiJ mice were obtained from our breeding colony. All animals were kept at 24 °C on a 12-h light and 12-h dark cycle and were provided with food and tap water ad libitum. All procedures and protocols were performed in accordance with national and European (86/609/EEC) legislation for animal care and experiments (animal licences No. A87/05 and 8.87-50.10.46.09.016; RP Münster and LANUV Northrhine-Westfalia). The sex chromosomal complement of the animals was confirmed by fluorescence in situ hybridization as published previously (Lewejohann *et al.*, 2009). Animals were anaesthetized with CO_2 before they were killed by cervical dislocation. Trunk blood was sampled and testes were dissected and fixed immediately in Bouin's solution for 24 h.

Analysis of the blood vessel/testis ratio

The fixed mouse testes were embedded in resin TV7100 (Hareus Kulzer, Wehrheim, Germany), cut into 3-μm-thick sections and PAS stained as described previously (Lewejohann *et al.*, 2009). The slides were analysed using an Olympus microscope with a camera attached. At a 10-fold magnification all areas of the entire testis cross section were photographed. The software Photoshop CS2 (Adobe Systems, San Jose, CA, USA) was employed to assemble the individual pictures into a combined

organ-wide overview and to embed the scaling from the microscope.

In the micrographs, the whole testes area and blood vessels were repainted on separate layers. Only blood vessels with a roundish appearance were marked. Along the scaling bar, an area of 100 μm × 1 pixel was marked and the pixel count of an area of 100 μm² was calculated. The repainted areas of the testes and blood vessel layers were analysed for their pixel count using the software's histogram function and transferred into metric units. Finally, the ratio of combined blood vessel's area in relationship with the whole testes area was calculated. The evaluation procedure is illustrated in Fig. 1.

Statistics

Data are presented as mean ± standard deviation (SD) and were analysed by Student's *t*-test or one-way ANOVA followed by Tukey's post hoc test with *p*-values corrected for multiple comparisons. All calculations were performed with GraphPad Prism version 6.02 for Windows (GraphPad Software, San Diego, CA, USA). Values were considered significantly different if $p < 0.05$.

RESULTS

Comparison of serum and testicular hormone levels in patients

Clinical parameters and serum hormone levels were compared between KS and SCOS patients and controls (Table 1, Fig. 2). KS patients were younger and had the smallest testicular volume. LH and FSH were significantly higher in KS and T, as well as free T, lower than in controls, while SHBG was comparable between all groups.

Table 1 Age, testicular volumes and serum hormone levels in Klinefelter Syndrome (47,XXY) and Sertoli-cell-only syndrome (SCOS) patients as well as controls[a]

	47,XXY ($N = 11$)	SCOS ($N = 30$)	Controls ($N = 9$)
Age (years)	28.7 ± 7.5	34.7 ± 5.0**	41.4 ± 3.0***
Bi-testicular volume (mL)	8.4 ± 4.5	27.3 ± 11.8***	56.9 ± 14.8***
FSH (IU/L)	26.1 ± 10.5	22.0 ± 7.2	4.6 ± 2.5***
LH (IU/L)	15.7 ± 5.3	7.4 ± 2.7***	3.5 ± 1.1***
Testosterone (nmol/L)	10.2 ± 5.2	12.7 ± 4.1	15.5 ± 4.0*
SHBG (nmol/L)	31.6 ± 14.0	31.3 ± 13.4	32.3 ± 11.5
Free testosterone (pmol/L)	209 ± 107	269 ± 73	334 ± 93**

SHBG, sex hormone-binding globulin. [a]Data presented as mean ± SD. Significant differences from KS patients marked: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

Concerning testicular hormones, ITT was the highest in KS when analysed per gram of tissue and comparable with both other groups when calculated per whole testes (Table 2, Fig. 2E, F). In contrast, SHBG per gram of tissue was not significantly different between groups, but total testicular SHBG was lower in KS than in SCOS and controls (Table 2).

The ratio of ITT/serum T (Fig. 2H) was markedly different, resulting in ~3800, ~1600 and ~400 in KS, SCOS and controls respectively. In contrast, the ITT/LH-ratio was comparable between all groups (Fig. 2G). Ratios of ITT/SHBG were significantly and progressively lower by one order of magnitude from KS over SCOS to controls, owing to an overall pronounced excess of ITT over SHBG in molar terms (Table 2).

Histological evaluations in patients

Both KS and SCOS patients had significantly reduced mean diameters of seminiferous tubules compared with controls

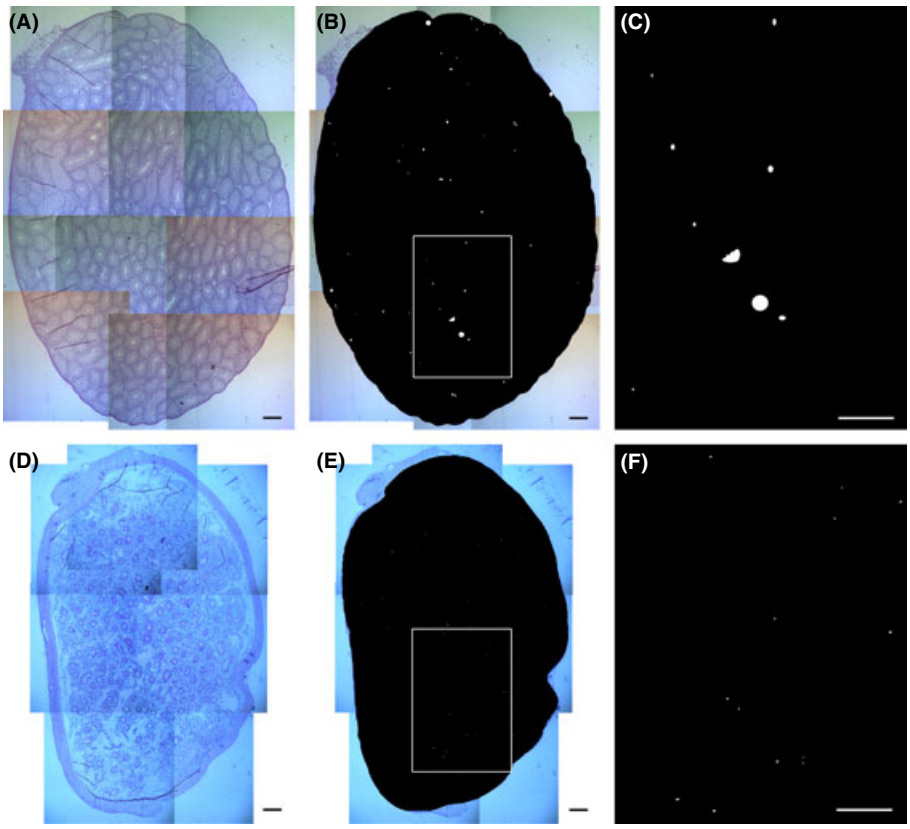


Figure 1 Evaluation of testicular cross sections of 40,XY* (A–C) compared with 41,XX^{Y}* mice (D–F) for areas covered by blood vessels. Assembling of testis pictures allowed assessing the entire organ area (A, D). Black labelling of the area covered by the testicular tissue and repainting of roundish blood vessels in white colour for size measure (B, E), which was done in higher magnification (C, F). Scale bars indicate 100 μm.

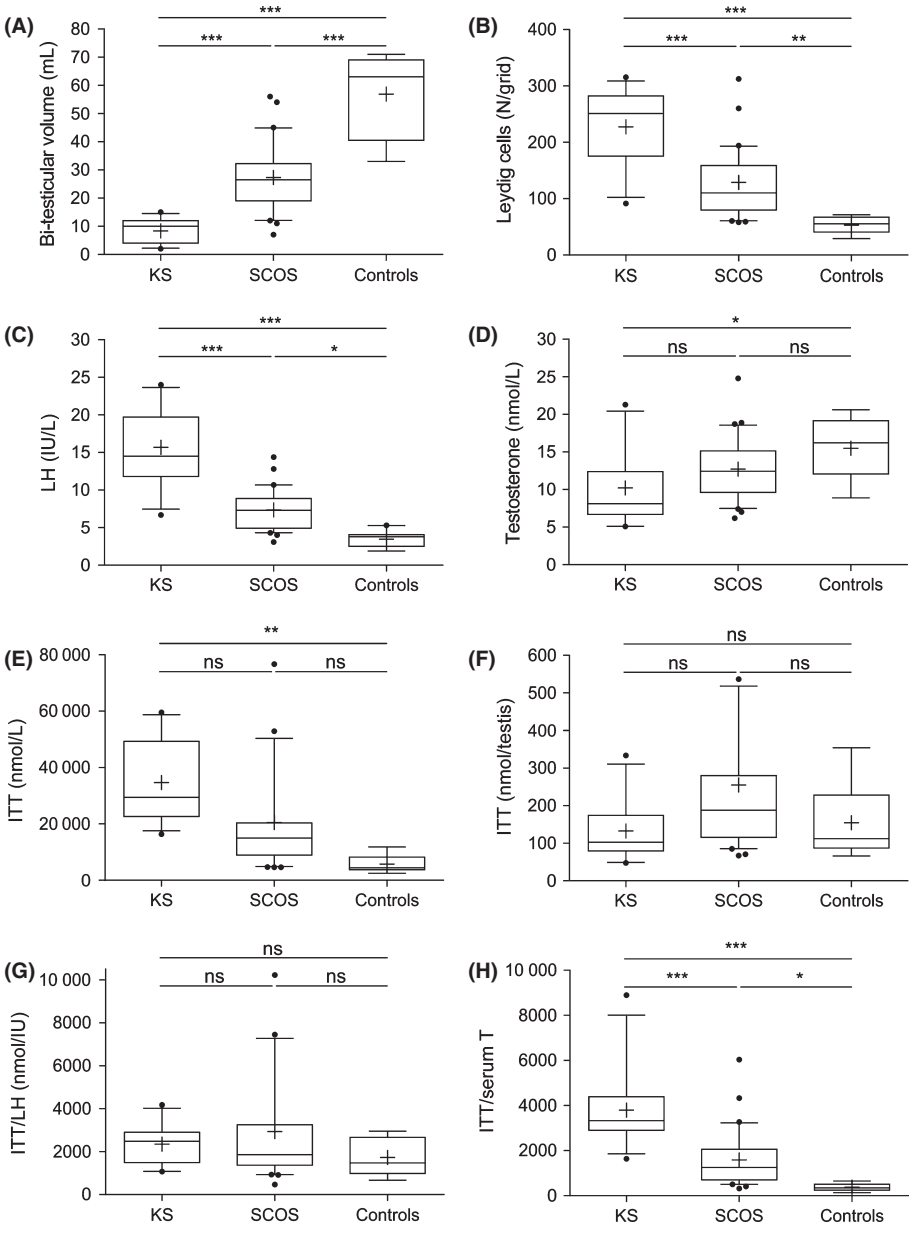


Figure 2 Box-and-whisker plots of selected clinical and testicular parameters (A–F) and calculated ratios of ITT/LH (G) and ITT/serum testosterone (H). Boxes contain 50% of the distribution, whiskers extend to 10–90%; lines in boxes represent the median, crosses the mean. Significant differences marked with $*p < 0.05$, $**p < 0.01$, $***p < 0.001$. KS, Klinefelter syndrome; SCOS, Sertoli-cell-only syndrome.

(Table 2). The tubule wall thickness was increased almost twofold in KS compared with controls. Leydig cell numbers were increased fourfold and twofold compared with controls and SCOS, respectively. However, Sertoli cell numbers per tubule were not different between KS and controls, but higher in SCOS than in both other groups.

Testis weight and serum testosterone in mice

Testes were 10-fold smaller in XX^{Y*} mice compared with XY* littermates (19.5 ± 3.7 mg vs. 196.8 ± 10.2 mg, $p < 0.001$, Fig. 3A). Serum T appeared to be lower in XX^{Y*} mice compared with XY* littermates, although significance was not reached ($n = 5$, 9.5 ± 4.2 mg vs. 22.6 ± 27.3 mg, $p = 0.32$, Fig. 3B).

Distribution of blood vessels in mouse testes

On the organ-wide overview of the testes, areas of all single blood vessels were analysed to gain their individual pixel numbers. The areas covered by blood vessels within the testes were significantly lower in XX^{Y*} (262.8 ± 143.8 μm²) than in XY* (906.9 ± 369.9 μm², $p < 0.01$) mice. The blood vessel/testes surface ratio correcting for the smaller testes of XX^{Y*} mice also revealed a significant reduction in XX^{Y*} ($1.5 \pm 0.3‰$) compared with controls XY* ($4.1 \pm 1.3‰$, $p < 0.01$, Fig. 3C). In addition, the distribution of blood vessel size was significantly different; especially larger blood vessels covering an area of more than 867 μm², which comprised 13% of all vessels and occupied 55% of the total vessel area in XY* mice, were not found in XX^{Y*} males (data not shown).

DISCUSSION

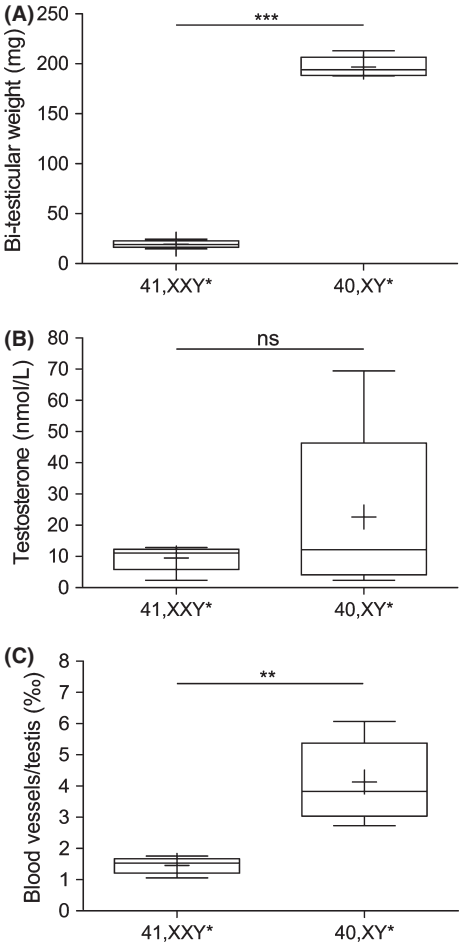
In this work, we demonstrate for the first time that ITT concentrations are significantly increased in men with KS, despite reduced serum T levels. This finding is in agreement with previous studies conducted in our KS mouse model. Male mice of the karyotype 41,XX^{Y*} have been demonstrated to resemble features of human KS adequately (Lewejohann *et al.*, 2009; Wistuba

Table 2 Testicular hormone levels and histological parameters in Klinefelter Syndrome (47,XXY) and Sertoli-cell-only syndrome (SCOS) patients as well as controls[a]

	47,XXY (N = 11)	SCOS (N = 30)	Controls (N = 9)
ITT (nmol/L)	34 703 ± 14542	20 515 ± 21917	5749 ± 3343**
ITT/serum testosterone	3793 ± 1857	1584 ± 1254***	375 ± 170***
ITT/LH (nmol/IU)	2353 ± 965	2932 ± 2845	1728 ± 857
ITT (nmol/testis)	132.8 ± 84.0	255.2 ± 265.4	154.5 ± 106.5
SHBG (nmol/L)	0.230 ± 0.181	0.396 ± 0.302	0.365 ± 0.354
ITT/SHBG	2.1×10^8	1.2×10^{7}**	2.2×10^{6}*
SHBG (pmol/testis)	1.2 ± 1.4	5.3 ± 4.4*	8.1 ± 6.1**
Tubule diameter (μm)	91.2 ± 54.2	112.4 ± 28.6	173.5 ± 23.9***
Tubule wall thickness (μm)	12.4 ± 3.1	9.5 ± 4.0	6.8 ± 1.4**
Leydig cells (N/grid)	227.4 ± 68.3	128.9 ± 59.2***	53.1 ± 14.6***
Sertoli cells (N/tubule)	25.8 ± 14.0	44.4 ± 16.7**	20.8 ± 3.8

SHBG, sex hormone-binding globulin; ITT, intratesticular testosterone. [a]Data presented as mean ± SD. Significant differences from KS patients marked: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

Figure 3 Box-and-whisker plots of testicular weight (A), serum testosterone (B) and relative testis section area covered by blood vessels (C) in 41,XX^{Y}* compared with 40,XY* mice (n = 5 per group). Boxes contain 50% of the distribution, whiskers extend to 10–90%; lines in boxes represent the median, crosses the mean. Testes were smaller, serum testosterone was lower, but not significantly different in 41,XX^{Y}*. However, relative blood vessel area was significantly reduced in 41,XX^{Y}* (** $p < 0.01$).



et al., 2010). Although impaired steroidogenesis had been suggested to be involved in the lowered serum T values observed in KS, Leydig cells were found to be hyperactivated when tested under hCG stimulation in vitro, and ITT values became similar to those of control animals, indicating an alternative cause for the relatively low serum testosterone (Wistuba *et al.*, 2010). Fittingly, Nieschlag *et al.* (1979) found a greater response to hCG stimulation in men with Leydig cell hyperplasia (but without KS) in vitro, which was not reflected by higher T levels in peripheral blood. In this study, we did find a lower mean serum T, which, however, was not significantly different from control mice, most likely because of the heterogeneity of the small sample size. When we analysed ~30 animals per group, we showed that serum T is significantly lower in XXY* mice compared with XY* (5). Because the current analysis was merely done to corroborate our previous findings and the XX^{Y}* mice are a very valuable resource, we did not see the need to repeat this experiment.

It was suggested that either T is bound in the testis by a disturbed ABP/androgen balance or the transport from the testis into the circulation might be hampered for unknown reasons. In this study, we showed that intratesticular SHBG, corresponding to mouse ABP, was very low compared with ITT in all men analysed and significantly decreased in KS patients when calculated per whole testis. Therefore, our present data do not support a role for testicular SHBG/ABP, while reduced release of T into the vascular tree remains a possibility. Using the KS mouse testis as a model, we observed a significantly smaller area to be covered by blood vessels, especially because of lack of large vessels.

The ITT concentrations in control men found in this study are similar to those found in previous studies based on fine needle testicular aspiration (Jarow *et al.*, 2001, 2005) or biopsy (Matthiesson *et al.*, 2005; Zamrazilova *et al.*, 2012). Those studies demonstrated that intratesticular DHT (which cross-reacts with T in our assay) concentrations are less than 1/100 compared to T, so we assume a minimal confounding effect of DHT in our study. When compared to testicular SHBG concentrations, ITT concentrations are about 10^6 (in controls) to 10^8 (in KS) times higher in molar terms, supporting the concept that testicular SHBG cannot function primarily as a binding protein for the testosterone produced locally (Jarow *et al.*, 2005). This is consistent with the view that testicular SHBG/ABP, if expressed at all, does not play any androgen-binding role in the human testis (Hammond, 2011).

Our data show that total ITT per testis does not change significantly, so that both SCOS and KS testes produce overall normal amounts of T, probably through an enrichment of fully functional Leydig cells. KS is long thought to be associated with a true Leydig cell hyperplasia, but so far data from stereological analyses are limited because of the limited access to patient material and biopsies do not allow an unbiased examination. However, that a supernumerary X chromosome provokes a true Leydig cell hyperplasia was experimentally shown in the 41,XX^{Y}* mouse model (Wistuba *et al.*, 2010; for review see Wistuba, 2010). Also in this study, it was not possible to determine the absolute number of Leydig cells per human testis, but the relative number of Leydig cells increased progressively in SCOS and KS patients. In analogy with the previous observation in KS mice, where stereological quantification was possible and both relative and absolute increase in number of Leydig cells was demonstrated (Wistuba *et al.*, 2010), we may conclude that overall

normal testicular T production is ensured by a higher number of Leydig cells. Nevertheless serum T is generally reduced in KS patients (and to a lesser extent in SCOS patients). In our normal controls, ITT is concentrated almost 400 times more than serum T, in agreement with previous studies (Jarow *et al.*, 2005; Matthiesson *et al.*, 2005), and this ratio rises to ~1600 in SCOS and to 3800 in KS patients. This suggests that in KS, but also in SCOS, T remains trapped in the testis.

Increased ITT concentrations in the presence of significantly increased serum LH levels and slightly decreased serum T levels suggest that the Leydig cells of KS patients respond correctly to LH by producing elevated T amounts, which, however, are hardly able to sustain normal peripheral levels. Therefore, the testosterone deficiency in KS patients is neither owing to reduced Leydig cell T production, nor to ineffective LH action, as previously demonstrated in mice (Wistuba *et al.*, 2010; Munir *et al.*, 2012). This accumulation of T within the testis is intriguing. As testicular SHBG (or ABP) is decreased in KS patients (despite normal numbers of Sertoli cells), a role for this Sertoli cell product to locally store T seems unlikely. The decrease of intratesticular SHBG, despite elevated FSH, could be a consequence of local androgen excess, as ABP expression is increased in testes from SCARKO mice, in which the androgen receptor is selectively knocked out in Sertoli cells (Tan *et al.*, 2005). Unfortunately, because of the very limited testicular material available, we were not able to analyse additional markers of Leydig cell function (e.g. INSL3).

In another interesting mouse model, the PTM-ARKO, in which the androgen receptor was selectively knocked out in the peritubular myoid (PTM) cells, ITT was very significantly increased, in spite of normal serum T levels (Welsh *et al.*, 2009). A progressive loss of spermatogonia occurs in this mouse model, demonstrating that both androgen action on PTM cells and stromal–epithelial interactions are fundamental for normal spermatogenesis. In the absence of this, T is produced but does not leave the testis. Taken together, these observations suggest that the defective secretion of T from the testis into the bloodstream may be androgen dependent via PTM cell function.

Taking advantage of our KS mouse model in search of an explanation, we analysed whether changes in the testicular vasculature might be involved in ITT sequestration. A reduction of vascular area was observed in KS mouse testes. However, we cannot discriminate whether this decreased blood supply is the cause of reduced T discharge in blood or the consequence of depopulated seminiferous tubules. In seasonal breeders, the testis undergoes growth and regression throughout the year, with loss of spermatogenic activity during the quiescence period (Meachem *et al.*, 2005). The regression of the seminiferous tubules is correlated with changes in the microvasculature in photoperiodic species such as the hamster (Mayerhofer *et al.*, 1989) and the cat (Alexandre-Pires *et al.*, 2012). The reduction in testicular vasculature area observed in KS mice might be one architectural consequence of germ cell depletion, as conceivably the energetic requirements of seminiferous tubules depleted of germ cells can be fulfilled by a lower blood supply. If this is correct, a lower vasculature extension/complexity should be observed in other animal models with reduced spermatogenesis and/or SCOS, a hypothesis to be explored in future studies. In humans, reduction of artery dimensions in different body areas has been reported in men with KS as compared to eugonadal controls (Foresta *et al.*, 2012), but it is not known whether the lower vasculature area observed in KS mice is also present in human KS testes. An alternative route for testicular T to leave the testes is the lymphatic system, which should be the scope of future research.

Previous anatomical and ultrastructural studies demonstrated special features of the microvasculature of the 'normal' human testis. Human Leydig cells are in close contact with both arterial and venous capillaries lined by a continuous non-fenestrated endothelium and basal lamina. Endothelial cells are rich in transcytotic vesicles, believed to be involved in the transport and exchange of hormones and metabolites (Ergün *et al.*, 1996). T release into circulation must occur at this level. It is usually assumed that T released from the endoplasmic steroidogenic reticulum diffuses freely through lipophilic cell membranes into the interstitial fluid and then into blood (Rommerts, 2004). However, a recent experiment showed that decapsulated testes from XXY mice cultured in vitro released the same amount of T into the medium as XY testes, despite significantly higher testicular content of T, and T concentration in the testicular venous effluent was threefold lower than in controls (Munir *et al.*, 2012). This raises the possibility that some as yet unknown factor, e.g. an active transporter, might be involved in T release from Leydig cells in the interstitial fluid and is impaired in KS testis. The involvement of MDR1, a member of the ABC transporter superfamily, in the transport of cortisol and aldosterone through plasma membranes was suggested about two decades ago (Ueda *et al.*, 1992). Mutations of ABCD1, another member of the ABC superfamily, are responsible for X-linked adrenoleukodystrophy (OMIM 300100), a disorder of the peroxisomal beta-oxidation of saturated very long fatty acids with a composite phenotype involving adrenal and gonadal failure (Brennemann *et al.*, 1997). Finally, the rat *Abca5* transporter is expressed in Leydig cells, with a putative role in intracellular steroid trafficking (Petry *et al.*, 2006). Future studies should therefore explore whether steroid transporters are involved in the sequestration of T in the testis.

Whether and how our findings might be linked to germ cell loss typical of KS remains to be analysed. In rodent models, a direct relation between testicular blood vessel supply and the localization of spermatogonial stem cells was shown (Yoshida *et al.*, 2007). Therefore, speculation may go along two routes: A disturbed angiogenesis may affect the SSC niches and thus contribute to the germ cell loss or, vice versa, the germ cells are lost because of other causes – most likely meiotic incompetence in KS – and the subsequent changes in testicular architecture adversely influence the vascular situation in the testicular microenvironment. This issue is highly relevant and could be addressed in the available mouse models, which is, however, outside the scope of this study.

In summary, the results of this study show that testicular T production per se is not impaired in men with KS. On the contrary, ITT concentrations are increased, as previously demonstrated in mouse models. This increase, however, does not appear to be due to heightened SHBG/ABP activity. The data from the KS mouse model let us speculate that a reduced vascular bed might be involved in impaired release of T into the bloodstream, although this could be a general phenomenon common to aspermatogenic testes. We consider our data a preliminary report which should stimulate future studies exploring the possibility that T is actively retained inside the Leydig cells by an as yet unknown mechanism.

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AUTHORS CONTRIBUTION

F.T. and M.B. performed the clinical research and O.S.D. and C.M.L. performed the mouse studies. F.T., M.S., O.S.D. and J.W. analysed the data and wrote the manuscript. S.K. performed the human biopsies and contributed clinical data including histology and testicular tissue. M.S. and J.W./J.G. designed and supervised the clinical and mouse parts of the study respectively. M.S. and J.W. together with E.N. conceived this study and accompanied it in all phases including review of the manuscript. All authors read and approved the manuscript.

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DISCLOSURE STATEMENT

The authors have nothing to disclose.

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