New approaches to the Klinefelter syndrome

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Abstract

The Klinefelter syndrome (KS), with an incidence of 1 to 2 per 1000 male neonates, is one of the most frequent congenital chromosome disorders. The 47,XXY karyotype causes infertility, testosterone deficiency and a spectrum of further symptoms and comorbidities. In recent years, significant progress has been made in the elucidation of the pathophysiology and the treatment of the KS. It became clear that, to a large extent, the clinical picture is determined by gene dosage effects of the supernumerary X-chromosome. The origin of the extra X-chromosome from either the father or the mother influences behavioural features of patients with KS. The CAGn polymorphism of the androgen receptor, located on the X-chromosome, has a distinct impact on the KS phenotype. KS predisposes to the metabolic syndrome and its cardiovascular sequelae, contributing to the increased mortality of patients with KS. Neuroimaging studies have correlated anomalies in brain structures with psychosocial problems.

The unexpected possibility to produce pregnancies and live birth with either ejaculated sperm – about 8% of KS men have a few sperm in semen – or with sperm extracted from individual tubules obtained by testicular biopsy can be considered a breakthrough. Testosterone substitution requires further optimisation in terms of when to initiate therapy and which preparations and dosages to use. Recently developed animal models help to further elucidation the genetic and pathophysiological basis and may lead to new therapeutic approaches to KS.

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Résumé

Le syndrome de Klinefelter (KS) avec une incidence de 1 à 2 pour mille nouveaux-nés de sexe masculin, est l’un des désordres chromosomiques congénitaux les plus fréquents. Le caryotype 47,XXY entraîne une infertilité et un déficit en testosterone et un éventail d’autres symptômes et co-morbidités. Au cours des dernières années, des progrès significatifs ont été faits dans l’élucidation des mécanismes physiopathologiques et le traitement du KS. Il est clair aujourd’hui que, dans une large mesure, le tableau clinique est déterminé par un effet dose-gène sur le chromosome X surnuméraire. L’origine paternelle ou maternelle de cet extra-chromosome X influence le comportement des patients avec un KS. Le polymorphisme CAGn du récepteur des androgènes, situé sur le chromosome X, a un impact différent sur le phénotype du KS. Le KS prédispose au syndrome...
métabolique et aux complications cardiovasculaires, contribuant à une mortalité accrue chez les patients avec un KS. Les études neuro-imageries ont montré une corrélation entre les anomalies de la structure cérébrale et les difficultés psycho-sociales. La possibilité inattendue d’obtenir des grossesses avec naissance vivante à partir, soit d’un sperme éjaculé — environ 8% des KS ont quelques spermatozoides dans l’éjaculat — soit avec du sperme extraï à partir des tubules individuelles obtenues après biopsie testiculaire peut être considérée comme une avancée. La substitution en testosterone nécessite encore des optimisations en termes d’âge auquel initier le traitement, de type de préparation et de dosage. Récemment, le développement de modèles animaux a aida à éclaircir plus avant les bases génétiques et physiopathologiques de ce syndrome ce qui permet d’envisager de nouvelles approches thérapeutiques du KS.

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1. Frequent syndrome, often overlooked

The Klinefelter syndrome (KS) – first described in 1942 [1] – is the most frequent type of congenital chromosomal disorder in males and is caused by aneuploidy of the sex chromosomes. Primarily, the KS is considered a disturbance of the exocrine and endocrine function of the testes. Indeed, infertility and testosterone deficiency are the key symptoms directing the patient either to the endocrinologist, to the andrologist or to fertility centres. However, beyond these symptoms of hypogonadism the KS is associated with a number of comorbidities and increased mortality of the affected men compared to the general population [2–5] (Table 1).

It is suspected that these comorbidities arouse the physicians’ attention more frequently than direct symptoms of hypogonadism, but the underlying disorder remains unrecognized and only the comorbidity is treated [6,7]. The proper diagnosis having been made, until recently comorbidities were considered primarily a consequence of the lack of testosterone. However, evidence is emerging that features such as increased height, metabolic syndrome, osteoporosis, breast abnormalities, thromboembolism, psychosocial and neurologic problems may also be caused by accompanying chromosomal and genetic abnormalities.

Over the past decade, several reviews have aimed to improve the level of information about this multifaceted syndrome and to increase the diagnostic frequency of this disease entity [7–10]. In the current paper we refer the reader to this general information about the KS and highlight here areas where significant progress has recently been made.

2. New genetic findings

2.1. X-chromosome inactivation and gene dosage effects

It becomes increasingly obvious that the clinical picture of KS patients is caused by gene dosage effects of the supernumerary X-chromosome. Similar to women, the additional X-chromosome in KS patients is silenced due to the process of X-inactivation [11–14]. One key player within this process is the non-coding RNA XIST, which spreads over the entire X-chromosome that becomes inactivated in cis and leads to heterochromatization and gene-silencing [15]. The methylation level of the CpG-island within the XIST promotor region can give evidence about the X-chromosome inactivation status.

In KS patients, it was shown that the XIST DNA-methylation level was comparable to women, but significantly different from men, indicating correct X-chromosome inactivation [14]. Other studies further demonstrated expression of the XIST gene in blood lymphocytes [12]. As approximately 15% of the X-linked genes escape the process of X-inactivation [16,17], these genes can be considered as the genetic background of KS [18].

These genes that are expressed from the active and the inactive allele are located across the entire X-chromosome, but are predominantly located in the pseudo-autosomal region (PAR), where recombination between the X and Y chromosome takes place [19]. Especially the escape of genes located in the pseudo-autosomal region of the X and Y chromosome (PAR) is of interest in KS patients as this causes the presence of three active copies. The escape of the PAR genes SHOX, PCGH11XY and SYBL1 was demonstrated epigenetically in blood lymphocytes of KS patients [14,20]. It is speculated that this ‘female’ expression pattern in e.g. Leydig cells, which normally only have a 46,XY karyotype, compromise their function. Nevertheless,

### Table 1

<table>
<thead>
<tr>
<th>Comorbidity</th>
<th>Incidence, %</th>
<th>Ref.</th>
<th>Mortality (SMR[^a^])</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gynecomastia</td>
<td>38</td>
<td>[8]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breast cancer</td>
<td>0.3</td>
<td>[2]</td>
<td>29</td>
<td>[2]</td>
</tr>
<tr>
<td>Thrombosis</td>
<td>4.7</td>
<td>[5]</td>
<td>8</td>
<td>[3]</td>
</tr>
<tr>
<td>Pulmonary embolism</td>
<td>2.3</td>
<td>[5]</td>
<td>6</td>
<td>[3]</td>
</tr>
<tr>
<td>Metabolic syndrome</td>
<td>44</td>
<td>[110]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type 2 diabetes</td>
<td>10</td>
<td>[110]</td>
<td>6</td>
<td>[3]</td>
</tr>
<tr>
<td>Erectile dysfunction</td>
<td>25</td>
<td>[111]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Osteopenia</td>
<td>40</td>
<td>[23]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Osteoporosis</td>
<td>10</td>
<td>[33]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hip fracture</td>
<td>?</td>
<td></td>
<td>39</td>
<td>[3]</td>
</tr>
<tr>
<td>Maldescended testes</td>
<td>27</td>
<td>[8]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mediastinal tumors</td>
<td>0.4</td>
<td>[5]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epilepsy</td>
<td>5.5</td>
<td>[5]</td>
<td>7</td>
<td>[3]</td>
</tr>
<tr>
<td>Mental retardation</td>
<td>4.2</td>
<td>[5]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Delayed verbal development</td>
<td>40</td>
<td>[112]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Language disorder</td>
<td>70–80</td>
<td>[68]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Legasthenia</td>
<td>50–70</td>
<td>[68]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Learning difficulties</td>
<td>75</td>
<td>[112]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADHD</td>
<td>63</td>
<td>[67]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Autism</td>
<td>48</td>
<td>[113]</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

[^a^] SMR: standardized mortality rate, i.e. predicted deaths [3].
complete inactivation and escape of X-chromosomal genes has to be proven in the tissue of interest, as expression/methylation in blood might not reflect the tissue-specific inactivation pattern. It was shown that in female cells, many of these genes show inter-individual variation of expression and also tissue-specificity [21,22].

The only study so far examining the KS methylomic and transcriptomic profile not only in the blood is a case report analyzing the prefrontal cortex and cerebellum of a KS patient [23]. Numerous autosomal regions with a different DNA-methylation pattern compared to male and female controls were found as well as tissue-specific expression differences of autosomal and X-chromosomal genes. Regarding genes escaping from X-inactivation, no consistent pattern of transcription was found, some of the genes were upregulated in the KS patient, others were down-regulated. Thus, a hint was found that alterations in gene expression of those genes normally escaping X-inactivation in females exist in KS patients and furthermore, that many autosomal genes were altered. It can be concluded that the phenotypic variation in KS patients can arise by autosomal alterations and the extent to which genes escape X-inactivation. Nevertheless, it is important to keep in mind that these data derive from a single case report. In addition to genes escaping from X-inactivation, the high phenotypic variation of the syndrome is also explained by other genetic and epigenetic effects such as parental origin of the X-chromosome, genetic polymorphisms and skewed X-inactivation of the X-chromosome.

2.2. Parental origin of the supernumerary X-chromosome

In KS patients, the extra X-chromosome can be inherited from both parents. In approximately 50% of the cases, it originates from the father due to non-disjunctions during meiosis I (MI). The paternal origin of the additional X-chromosome can only arise by MI errors as errors in meiosis II (MII) would lead to XXX or XYY zygotes. The other 50% are caused by maternal errors that are variable in origin: failure during maternal MI account for 48% of maternal XXX, in 29% a failure during MII occurs and in 7% of the maternal cases, the origin of the error remains unclear. In addition 16% of the maternal cases can arise from errors in early mitotic divisions of the zygote [24].

The parental origin of the supernumerary X-chromosome has been suggested to play a pivotal role in clinical features of KS [10,25]. The presence of two identical or two different X-chromosomes might possibly interfere with the fine-tuned process of X-chromosome inactivation. Especially for the variability of the KS phenotype parent-of-origin (of the extra X-chromosome) effects have been proposed. Parent-of-origin effects have been demonstrated regarding cognition and behavioural aspects. KS patients with a maternally inherited X-chromosome showed higher scores on schizotypal traits [26]. Another study also showed an influence of the parental inheritance of the supernumerary X-chromosome on the variation of the cognitive phenotype in KS patients by demonstrating more frequent speech and language problems in KS patients with a paternally inherited extra X-chromosome [27]. However, other studies do not support this hypothesis [28,29]. It further remains unknown if the inheritance of the supernumerary X-chromosome accounts for other phenotypic differences, e.g. infertility or the metabolic syndrome.

2.3. The androgen receptor CAGn polymorphism

The androgen receptor, which is located on the X-chromosome, contains a highly polymorphic trinucleotid repeat (CAGn) in exon 1 [30]. The length of this repeat normally varies between 9 and 37 [31] and is correlated with physiological androgen effects. The CAG repeat length in KS patients causes phenotypical variation by affecting height, arm span, cholesterol, haematocrit and haemoglobin [18,32]. The positive correlation to height and arm span could possibly be related to a later onset of puberty with longer CAGn and thus later activation of the pituitary-gonadal axis [25]. Furthermore, Zitzmann et al. [32] found that long CAGn was predictive for gynecomastia and smaller testes, whereas KS patients with short CAGn had more stable partnerships and professions that required higher standards. Regarding the pharmacogenetic impact of the CAG repeat length, different findings exist: one study reports a suppression of LH, increase of haemoglobin and prostate growth in testosterone treated KS patients with longer CAGn [32], whereas no impact of CAGn on the effect of testosterone treatment in KS patients was found by Bojesen et al. [33].

2.4. Skewed X-inactivation

In females, X-inactivation is random, meaning the ratio of activation/inactivation is approximately 50%. In many studies it was reported that the ratio of activation/inactivation in KS patients was skewed to 80% with the preference of one allele. The percentage of KS men with skewed X-inactivation varies between 10 and 50% in different studies [9,18,28,34,35]. Data regarding an impact of CAG length on the preferably inactivated allele are contradictory: a preference of the shorter CAG allele was reported by Zitzmann et al. [36] whereas Suzuki et al. [37] reported a preference of the longer allele. Three studies found no preferential allele [9,18,38].

It is possible that an abnormal or skewed inactivation in men with a supernumerary X-chromosome could lead to reproductive or cognitive problems that are typically seen in this group because of inadequate inactivation of the genetic material or a selective allelic drop-out of certain X-chromosomal genes. Nonetheless, no differences in anthropometrical, hormonal and bone-related phenotypical features between KS patients with skewed inactivation and those with normal inactivation were found [18].

The different degrees of phenotypic manifestations in patients with KS were demonstratively shown in a monozygotic twin pair with a 46,XY/47,XXX mosaicism [39]. One brother had a hypo-gonadic phenotype with very low testosterone levels, whereas his twin sibling had no clinical sign of hypogonadism with normal testicular function and normal testosterone levels. Furthermore, the height between the monozygotic twins varied, one had normal mid-parental height, the other clearly exceeded mid-parental height. The key factor to the phenotypic differences
of these twins is most likely the degree of mosaicism in the different tissues, with the testicular tissue of case II showing a lower degree mosaicism than case I. The difference in the height, that is determined due to expression of the *SHOX* gene [40], might be explained by an overexpression of the *SHOX* gene in the taller brother.

3. Can men with KS be considered fertile?

Although about 8% of men with KS have sperm in their ejaculates [8,41], paternity has been reported only in very few cases [42–44] and until recently, KS patients were generally considered infertile. However, using ejaculated sperm [45–47] or sperm extracted from testicular biopsies (TESE) (e.g. [48]) and applied for direct intracytoplasmic injection into oocytes (ICSI) pregnancy and live-born offspring could be produced. While only few tubules in the testes display full spermatogenesis, these can be precisely located using microsurgical techniques [49]. A comparison between results from conventional TESE with micro-TESE shows that micro-TESE is superior in terms of sperm retrieval and live-born children [50] (Table 2).

Surprisingly, the children born show only a slightly higher rate of sex chromosome aneuploidy compared to newborns in general. This triggers the question how these children obtained a regular chromosomal set while their fathers were so-called “non-mosaic 47,XXY”. Indeed, karyotyping of sperm from Klinefelter men showed over 95% normal haploid spermatogonia [51,52]. How the euploid sperm arise from the testes of a Klinefelter man is an object of current debate. Sciurano et al. [53] demonstrated that these sperms obviously derive from isolated foci with euploid spermatogonia while somatic Sertoli cells have a XXY constellation. They speculate that the second X-chromosome may get lost in some spermatogonia during ontogeny and then continue to undergo meiosis to produce X- or Y-sperm.

Another currently discussed topic is the possible impact of age on spermatogenesis in Klinefelter men. Some investigators conclude from their findings that the chances of finding sperm in testicular biopsies are higher in younger than in older patients [54–56]. As a consequence, cryopreservation of testicular biopsy material from pubertal boys with KS before testosterone substitution is being considered for later use when paternity may be requested [57].

### 4. Clinical and metabolic features

Men with KS exhibit marked variations in phenotype, which may range from males with severe signs of androgen deficiency to normally virilised males [58]. KS men are significantly taller than normal men, but similar in height compared to other hypogonadal men, while 46,XX males fall in the range of normal women [59]. Another genetic finding contributing to height is the polymorphism of the androgen receptor: patients with longer CAG repeats of the AR are taller than those with short CAG repeats [36]. These phenomena may be explained by the assumption that growth controlling genes reside on the Y chromosome, not present in women and 46,XX-males resulting in smaller height, while lack of testosterone contributes to delayed closure of the epiphyses causing taller stature. However, the lack of Y-linked growth genes in the 46,XX-males may cause short stature despite low testosterone levels in the range of Klinefelter men.

Hypogonadism itself causes an unfavourable pattern of cytokines and adipokines, adversely altering the inflammatory status leading to premature vascular damage and metabolic disorders [60]. These mechanisms are also present in men with KS who have an even higher metabolic risk than other hypogonadal men since the incidence of diabetes type 2 is significantly higher in KS than in other forms of hypogonadism such as isolated hypogonadotropic hypogonadism [61]. KS is associated with significantly decreased insulin sensitivity and HDL cholesterol levels while total fat mass and LDL cholesterol, triglyceride, CRP and leptin are significantly increased [62]. As a consequence, the metabolic syndrome develops a complex patho-physiological disorder consisting of an accumulation of visceral adipose tissue, dyslipidemia, insulin resistance and hypertension. Men affected by this syndrome have a fivefold greater risk of developing type 2 diabetes mellitus and are three times as likely to suffer a heart attack or stroke compared with those not affected (Table 1). These risks are not restricted to men of Caucasian origin, but have also been confirmed in patients with KS in Japan [63] and in China [61].

Obviously, other factors than lack of testosterone must contribute to this phenomenon. One of such factors could be a generally reduced diameter of large and medium sized arteries in KS patients as revealed by ultrasonographic investigations [64]. As this has not been reported in other forms of hypogonadism, this could be a testosterone independent phenomenon specific for the KS. Whether left ventricular dysfunction [65] and other cardiovascular abnormalities [66] are related to reduced arterial parameters remains to be investigated.

### 5. Psychoneurologic function

The psychological, intellectual and social development of boys with KS may be completely normal. However, over half of the patients develop language disorders and legasthenia, causing difficulties in communication, in learning and in school (Table 1). In addition, memory and decision-making may be impaired and many boys develop symptoms of autism spectrum disorders (ASD) [67] as well mood and anxiety disorders [68].

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Success rates for sperm retrieval and newborns in patients with Klinefelter syndrome using conventional TESE or micro TESE (data from [50]).</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Publications</strong></td>
<td><strong>Patients (n)</strong></td>
</tr>
<tr>
<td><strong>TESE</strong></td>
<td>14 publications, 1997–2010</td>
</tr>
<tr>
<td><strong>Micro TESE</strong></td>
<td>8 publications, 2005–2012</td>
</tr>
</tbody>
</table>
This leads to behavioural and social problems and isolation. As a result, professional status and income may remain below the family status and below the population average [69]. Social maladjustment and isolation may also explain the higher rate of criminal offences among men with KS, especially concerning arson, sexual abuse and exhibitionism [70]. Furthermore, among 1205 prepubertal boys referred for suspected fragile X-syndrome because of mental retardation of unknown aetiology, eight patients with KS were discovered [71].

Neuroimaging studies have revealed anomalies in the brain structures of boys and adults with KS, which correlated with psychosocial problems. “There are increases in the grey matter volume of the sensorimotor and parieto-occipital regions, as well as significant reductions in amygdala, hippocampal, insular, temporal and inferior-frontal grey matter volumes. Widespread white matter abnormalities have been revealed, with reductions in some areas (including anterior cingulated, bilaterally) but increases in others (such as left parietal lobe)” [72]. These alterations may be caused by excessive expression of genes that lie in the pseudo-autosomal regions of the X-chromosome. These genes have homologies on the Y-chromosome, therefore in KS there are effectively three copies. The genes in this region are not subjected to X-inactivation although other regions of the X-chromosome will be subjected to X-inactivation as in normal females. Alternatively, there is evidence that up to 15% of other genes on the “inactivated” X-chromosome usually escape inactivation in normal females. They could therefore be expressed in excess in KS relative to typical men, but may not be expressed to the same extent as in typical 46,XX women [12,72].

6. Testosterone treatment

Patients with KS eventually need to be substituted with testosterone. Testosterone treatment is usually started when the levels fall below the normal range for adult males, mostly occurring in the middle of the third decade of life. According to clinical practice and current guidelines on treatment of hypogonadism substitution can be performed with injectable testosterone esters or transdermal testosterone gels for long-term substitution or, on a more temporary basis, by oral testosterone undecanoate capsules [73–75]. Since testosterone substitution has been administered since the KS was first described and long before the criteria of evidence-based medicine were introduced, no controlled trials have been performed aiming at identifying the most appropriate testosterone preparation and optimal dosing. Effects were evaluated by patients’ reports, clinical observation and laboratory findings. The clinical impression prevailed that testosterone substitution would alleviate most symptoms such as decreased libido, erectile dysfunction, anaemia, osteoporosis, lumbaro, depression, language abilities and social maladjustment. Autoimmune diseases [76,77] and leg ulcers [78] were found to improve under testosterone treatment. Numerous further uncontrolled studies and case reports confirm this impression. However, the patient and the empathic physician notice that this substitution is not optimal and does not fully normalise the patient’s life.

Only recently, more critical attention has been paid to the clinical outcome of testosterone substitution. For example, despite standard testosterone substitution, quality of life in Klinefelter patients has been found to be below the average of the general population [79]. Bone mineral density was found lower in adult Klinefelter patients than in age-matched controls despite testosterone substitution [33], while other studies confirm the beneficial effects of long-term testosterone substitution on bone health [80]. The increased height in males with KS (see above) may not be due to testosterone deficiency as has always been assumed and thus considered a marker of hypogonadism, but rather may be a consequence of an extra dosage of SHOX, an X chromosomal and Y chromosomal gene that may escape X chromosomal inactivation [81]. Similar to the role of the androgen receptor polymorphism in the development of the KS phenotype, the CAG repeat length also plays a modifying role in the biological effects of testosterone treatment [82,83]. Clearly, further controlled clinical trials are needed to fully assess the benefits of testosterone substitution and optimized treatment regimens.

Another very topical question is when and how testosterone treatment should be started. There were early observations that KS boys were better adjusted and had improved cognitive and learning abilities when they were substituted from early puberty onwards [84]. However, this never became a generally accepted concept and only recently has this question been taken up again in clinical trials (e.g. [85,86]), some of which are currently ongoing. It is also difficult for the endocrinologist to convince psychiatrists and social workers of the potential usefulness of this hormone in adjusting the possibly criminal adolescent to his environment when testosterone was considered an agent causing aggression and overlooking its real function in socialisation (e.g. [87]).

Taking into consideration that there may already be a testosterone deficiency before puberty in KS boys, some investigations even propagate testosterone substitution during infancy. In a retrospective study comparing 34 47,XXY-boys who had received short courses of testosterone treatment at ages three and six years with 67 non-treated KS patients, a significant advantage in cognitive, language, intellectual and neuromotor functions of the treated boys was observed [88]. These encouraging findings open a new dimension for testosterone substitution in KS patients.

7. Animal models: guides to new clinical approaches

Animal models are required to explore the disturbed genotype-phenotype correlations in addition to and beyond possible clinical investigations. Although comparable sex-chromosomal aberrations occur sporadically in several mammalian species (reviewed in [89,90]), quite generally resembling features also observed in human pathology, the main requirement for an animal model is that it can be continuously generated, that it resembles the phenotype of patients as closely as possible and that it is practicable within a reasonable work and cost load.

For the KS, this was particularly difficult as this sex-chromosomal disorder is associated with male infertility—thus
Fig. 1. Impaired memory in a mouse model of Klinefelter syndrome. In non-conditional learning experiments, 41,XXY* mice showed an impaired memory recognition. As a test system, the novel object task was used, which is based on the natural exploration behaviour of mice. A. In the test procedure, the animals are firstly exposed to a blank open field arena. B. In a second round they encounter two identical objects in the open field, of which one (C) is replaced by a novel object in the final test. The measure is the time spent in exploration of one or the other object. In mice with normal cognitive abilities, the novel object is preferred over the known one. D. When undergoing the novel object task, 40, XY* littermate control mice showed a normal preference for the novel object exploration, whilst the 41,XXY* males remained close to the 50% line (no preference) indicating a disturbed memory recognition ($P < 0.05$, unpaired Wilcoxon test, two tailed). The figure is based on a data set from Lewejohann et al., 2009 [96].

These mice have hypergonadotropic hypogonadism, are infertile due to germ cell loss, have altered body proportions, disturbed bone metabolism and exhibit cognitive and behavioural deficits, all hallmark features of the human disorder [90,98,99]. During recent years, these models have been used in experimental studies that provided important in-depth insights, some of which had already been confirmed in clinical settings and which have broadened understanding of the human disorder. For example, verbal and nonverbal/spatial cognitive deficits and neuropsychological alterations have been reported (i.e. language disorders, reading disabilities, reviewed in [100]) but it remained unclear to which extent these are intrinsic in genetic predisposition, due to the endocrine milieu or caused by socially enhanced learning problems of Klinefelter boys. The mouse models allowed experimental investigation of behavioral and cognitive conditions in males with a supernumerary X-chromosome [101] and confirmed that such males had learning deficits when exposed to a classic pavlovian setting, in which they, although able to fulfil the task, required much longer training than controls [94]. In a non-conditional test (novel object task), the 41,XXY* mice were shown to exhibit memory recognition problems [96] (Fig. 1); moreover, social preference was also altered in the 41,XXY model [98]. Interestingly, in a study in which the setting of the non-conditional testing of memory recognition, as performed in the 41,XXY* mice, was adapted for Klinefelter boys, the basic failure observed in mice was confirmed in these patients [102].
Disturbed bone metabolism, frequently observed in KS patients, was also addressed in the mouse model. Bone volume loss occurring in patients may be a consequence of testosterone deficiency and therefore treatable by testosterone replacement or may be due to other factors. 41, XXY male mice were used to analyse bone metabolism and remodelling under androgen treatment. It was clearly shown that testosterone deficiency alone did not fully explain the bone phenotype as reduced bone volume was also maintained in treated XXY mice exhibiting normal blood testosterone levels, as in humans [18], indicating a genetic component involved in disturbed bone formation possibly enhanced by the hypogonadal status [99].

Hypergonadotropic hypogonadism is a general feature of KS. Disturbed Leydig cell function or maturation was suggested as a likely reason for this endocrine phenotype, as testicular histology of males with a supernumerary X-chromosome commonly revealed Leydig cell hyperplasia [89]. Logically, it was proposed that the Leydig cells of Klinefelter patients should be functionally disturbed and therefore responsible for the endocrine failure. As this could not be proven in patient material, the issue was addressed in the 41, XXY* mouse model by analysing Leydig cells isolated from the testis. Surprisingly, when the expression profile of those cells was examined, the cells were found to be hyperactivated, i.e. all markers were over-expressed but no maturation defect could be observed. Similarly, the LH receptor was fully mature and responded to CG stimulation as expected and the testosterone response of the cells in vitro was superior to controls. In conclusion, the Leydig cells in males with a supernumerary X-chromosome appear to compensate as well as possible for the disturbed milieu. This was confirmed when intratesticular testosterone (ITT) was measured and found to be as high as in controls [97]. Arguing from these animal findings, an analysis of ITT levels in patients showed that also in the human ITT levels are not different from healthy men [103]. This discrepancy between lowered serum testosterone and normal ITT levels remains unclear. However, recently generally altered circulation in Klinefelter patients was reported [64] and it was hypothesized that diminished testicular vascularization might impede testosterone secretion. Following this hypothesis, the testicular vessels from 41, XXY* mice were analysed – such measurement is impossible in patient biopsies since the entire testis has to be evaluated – and it could be confirmed that the testicular blood supply is hampered in males with a supernumerary X-chromosome by an absolute reduction of area covered by vessels and in particular by a lack of those with a larger diameter [103].

The loss of the germ line is another general feature of the KS, also present in both mouse models with a similar developmental pattern, i.e. being complete at the onset of puberty. In mice, individuals with small foci of active spermatogenesis have been observed as seen in some patients undergoing TESE [53,104,105] but not adequately examined. The changes that finally result in the fading of the germ line already begin early in development. As access to material from Klinefelter boys is extremely limited, studies are restricted to sporadic observational reports [106]. Investigations in the mouse model showed that the germ line is already altered in utero and that those germ cells maintained beyond birth undergo some kind of euploid correction [92,93,107]. The euploidy of surviving germ cells in focal spermatogenesis of patients was confirmed recently and Sertoli cells – although of the XXY karyotype – were found to support gamete maturation, which is also true in 41,XXY mice when transplanted with healthy spermatogonia [53,95,104]. Additionally, analysis of the spermatogonial cell population in the postnatal phase, revealed that in the testsis of 41,XXY* mice expression and regulation of markers associated with stem cell features are disturbed, indicating an impaired stem cell potential of the spermatogonia. In sites with focal spermatogenesis, this deregulation is rescued [105]. These data have disproven the former assumption that aneuploid germ cells are not able to survive meiotic division and are therefore lost. It was rather demonstrated that an intrinsic disturbance of the germ line disables progression as long as there is no euploid correction. Altered vascularization might play a role as spermatogonial stem cells need close vicinity to testicular blood vessels [108]. It might be speculated that the testicular architecture and the endocrine phenotype are only consequences of genetically driven altered circulation – a thought which, once proven, could open novel therapeutic approaches. Finally, there is another important advantage provided by the mouse models. As the entire phenotype seen in KS must be due to gene dose effects from the supernumerary X-chromosome and as X-inactivation is not different from healthy females [14,81,109], the candidate genes responsible must be amongst those that escape from inactivation, the so-called escapee genes. While in humans about 15% of the X-linked genes escape from inactivation, in mice only 13 escape genes are known [22] – but these few candidates are sufficient to induce a phenotype exactly resembling that of patients. Exploring this small number of genes in various mouse tissues revealed an unexpected tissue- and gene-specific expression pattern, which was recently also confirmed in a patient [23,109].

Analysis of the expression patterns of these genes will allow important genotype-phenotype correlations as well as elucidation of gene function and regulation of the basic mechanisms behind this sex-chromosomal trisomy in the KS.

8. Conclusion and outlook

In recent years, tremendous progress has been achieved in our understanding of the pathophysiology and the clinical management of the KS. Foremost, the fact that a man with KS is no longer absolutely infertile, can be considered a breakthrough. Furthermore, it became clear that the KS is not simply a form of hypogonadism, but that infertility and lack of testosterone are only symptoms in a wider spectrum of other diseases and comorbidities. Recognition of this fact by the medical profession should contribute to enhanced diagnostic identification of this syndrome that has often been overlooked. While the regimen and benefits of testosterone treatment have not yet been fully evaluated, the importance of other therapeutic modalities are emerging, especially psychotherapeutic and behavioural support in childhood, adolescence and adulthood. These changes in the clinical management of men with KS are based on thorough
genetic investigations, which, together with recent findings from mouse models, will fuel further improvements for benefit of affected patients.

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

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