

# Novel genetic aspects of Klinefelter's syndrome

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**ABSTRACT:** Klinefelter's syndrome (KS) is the most common chromosome aneuploidy in males, characterized by at least one supernumerary X chromosome. Although extensively studied, the pathophysiology, i.e. the link between the extra X and the phenotype, largely remains unexplained. The scope of this review is to summarize the progress made in recent years on the role of the supernumerary X chromosome with respect to its putative influence on the phenotype. In principal, the parental origin of the X chromosome, gene-dosage effects in conjunction with (possibly skewed) X chromosome inactivation, and—especially concerning spermatogenesis—meiotic failure may play pivotal roles. One of the X chromosomes is inactivated to achieve dosage-compensation in females and probably likewise in KS. Genes from the pseudoautosomal regions and an additional 15% of other genes, however, escape X inactivation and are candidates for putatively constituting the KS phenotype. Examples are the *SHOX* genes, identified as likely causing the tall stature regularly seen in KS. Lessons learned from comparisons with normal males and especially females as well as other sex chromosomal aneuploidies are presented. In addition, genetic topics concerning fertility and counseling are discussed.

**Key words:** Klinefelter's syndrome / X chromosome / X inactivation / parental origin / sex chromosome aneuploidy

## Introduction

Klinefelter's syndrome (KS) was first described in 1942 (Klinefelter *et al.*, 1942), and the cause for the syndrome was later found in 1959 as a supernumerary X chromosome resulting in the karyotype 47,XXY (Jacobs and Strong, 1959). About 80–90% of KS cases bear this 'original' karyotype, whereas the remaining exhibit (in decreasing frequency) varying mosaicism (e.g. 47,XXY/46,XY), carry additional sex chromosomes (48,XXX; 48,XXYY; 49,XXXXY) or structurally abnormal X chromosomes (Bojesen *et al.*, 2003; Lanfranco *et al.*, 2004). In the late 1960s and early 1970s, six large surveys of consecutive newborns (summarized by Hook and Hamerton, 1977) among other chromosomal aneuploidies established the prevalence of KS as 1 per 1000 same sex births. Later studies found a higher prevalence of up to 1 in 500 boys (Nielsen and Wohler, 1990), and recently an increase in the prevalence of KS in opposition to the other sex chromosome trisomies (47,XYY males and 47,XXX females) has been described (Morris *et al.*, 2008). In any case, KS is the most common chromosomal aberration in men with 0.1–0.2% of the male population affected. When considering infertile men, the prevalence of KS is even much higher and increases from ~3% in unselected to ~13% in azoospermic patients (Van Assche *et al.*, 1996; Vincent *et al.*, 2002) which we recently confirmed in our patient cohort (Tüttelmann *et al.*, 2008; Tüttelmann and Nieschlag, 2009), making KS the most frequent genetic cause of azoospermia.

KS is regularly associated with hypergonadotropic hypogonadism and infertility due to azoospermia, but with marked variations in the phenotype (Lanfranco *et al.*, 2004). The 'prototypic' man with KS has traditionally been described as tall, with sparse body hair, gynecomastia, small testes and decreased verbal intelligence (Bojesen and Gravholt, 2007). Yet, the clinical picture of XXY males may range from severe signs of androgen deficiency, or even a lack of spontaneous puberty, to normally virilised males who only consult a doctor because of their infertility. This variability is most likely explaining why only 10% of KS men are diagnosed until puberty and only ~25% during their lifetime according to a large Danish registry study (Bojesen *et al.*, 2003) in accordance with an earlier report (Abramsky and Chapple, 1997).

The increased morbidity and mortality in KS (Bojesen *et al.*, 2004, 2006; Swerdlow *et al.*, 2005) underline the need for an early diagnosis of a larger proportion of KS and necessitate a more widespread screening. Unchanged, the gold standard for diagnosing KS remains karyotyping of metaphase spreads from cultured peripheral blood lymphocytes. The major benefit of karyotype analysis is the simultaneous evaluation of the chromosome structure with respect to translocations, inversions and deletions. Nevertheless, a suspected diagnosis of KS may be quickly corroborated by analysis of a buccal smear to detect Barr bodies (Kamischke *et al.*, 2003), i.e. the inactivated supernumerary X chromosomes (see below), but does not reach an adequate sensitivity to serve for screening (Pena and Sturzeneker, 2003). In the last

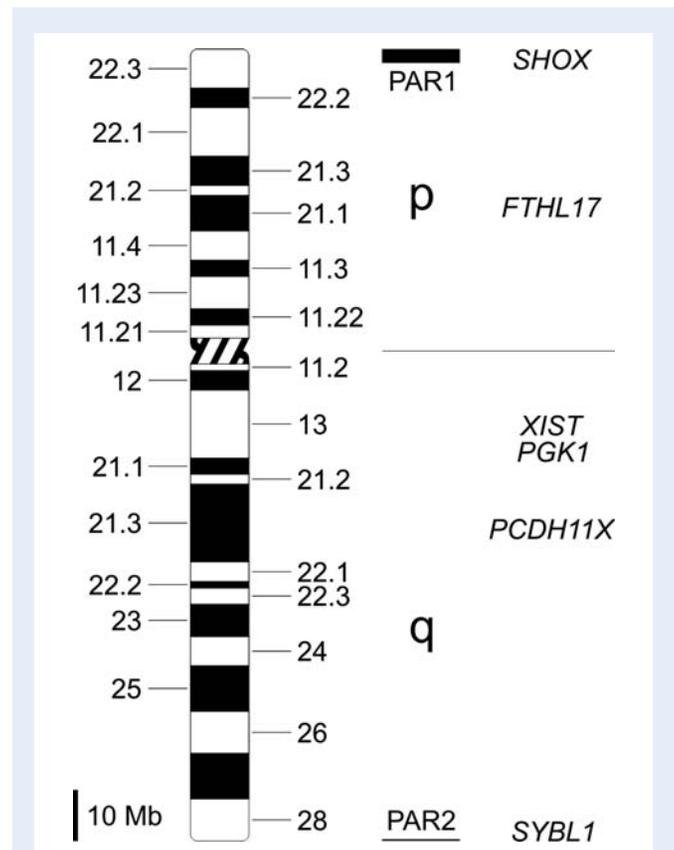
years, new screening methods have been published: fluorescence *in situ* hybridization (FISH) may be used to estimate mosaicism in more detail by analyzing a larger number of interphase nuclei (Abdelmoula *et al.*, 2004; Lenz *et al.*, 2005). The extra X can also be detected by quantitative real-time PCR (qPCR) of, for example, the androgen receptor (AR) gene (Fodor *et al.*, 2007; Ottesen *et al.*, 2007; Plaseski *et al.*, 2008) or array comparative genomic hybridization (array CGH, Ballif *et al.*, 2006). In comparison to karyotyping, the benefit of both methods is the lack of time-consuming and costly cell culture, and whereas qPCR can be considered a quick and inexpensive method, the main advantage of array CGH is the higher resolution of up to or even below 1 kb of altered DNA.

Although KS has been studied extensively in the last decades, the pathophysiology, i.e. the link between the supernumerary X and the phenotype, largely remains unclear and the variability unexplained. Apart from normal interindividual genetic variation, several genetic mechanisms may explain the variability of the phenotype, clinical features, life circumstances, life expectancy and fertility (Simpson *et al.*, 2003): in principal, the parental origin of the X chromosome, gene-dosage effects in conjunction with (possibly skewed) X chromosome inactivation (XCI) (Samango-Sprouse, 2001), and—especially concerning spermatogenesis—meiotic failure may play pivotal roles. Much knowledge was and will be gained from the available mouse models of KS (Lue *et al.*, 2001; Lewejohann *et al.*, 2009), which is dealt with in detail in a separate paper in this issue (Wvistuba, 2010). The scope of this review is to summarize, from a genetical viewpoint, the progress made in recent years on the role of the supernumerary X chromosome with respect to its putative influence on the phenotype.

## The human X chromosome, inactivation and gene-dosage

The human sex chromosomes (X and Y) originate from an ancestral homologous chromosome pair, which during mammalian evolution lost homology due to progressive degradation of the Y chromosome (Charlesworth and Charlesworth, 2005; Graves, 2006). In addition to specific regions, both sex chromosomes carry short regions of homology termed pseudoautosomal regions (PAR) as they behave like an autosome and recombine during meiosis (Helena Mangs and Morris, 2007). As depicted in Fig. 1, while PAR1 comprises 2.6 Mb of the short-arm tips of both X and Y chromosomes, PAR2 at the tips of the long arms spans a much shorter region of 320 kb (Freije *et al.*, 1992; Rappold, 1993). Since the human X chromosome has been almost completely sequenced, it became clear that (i) PAR1 contains at least 24 genes whereas in PAR2 only 4 genes were identified and that (ii) probably as many as 10% of X chromosomal genes are specifically expressed in the testis (Ross *et al.*, 2005).

Following Lyon's hypothesis (1961), one X chromosome is transcriptionally inactivated in somatic cells of females to equalize the dosage of X-encoded genes to that of male cells, consequently leading to cellular mosaicism for X-linked parental alleles. The inactive X had already been noted by Barr and Bertram (1949), termed Barr body, which can easily be stained from, for example, a buccal smear. XCI may occur randomly or by imprinting where the paternal X chromosome is silenced in the preimplantation embryo and extra-embryonic tissue. Random XCI occurs in the epiblast, inactivating



**Figure 1** Human X chromosome with G-bands (Giemsa stained), pseudoautosomal regions (PAR1/2) marked to scale and approximate position of exemplary genes described in the text.

*SHOX*, short stature homeobox; *FTHL17*, ferritin, heavy polypeptide-like 17; *XIST*, X (inactive)-specific transcript (non-protein coding); *PGK1*, phosphoglycerate kinase 1; *PCDH11X*, protocadherin 11 X-linked; *SYBL1* synaptobrevin-like 1 (also: *VAMP7* vesicle-associated membrane protein 7)

either the maternally or paternally inherited X chromosome (Ng *et al.*, 2007), resulting in an active and an inactive chromosome and this state is then stably transmitted to descendant cells (Kalantry *et al.*, 2009). The X-inactivation centre initiating XCI contains the X (inactive) specific transcript (*XIST*) encoding an untranslated RNA able to coat and silence the X chromosome. However, besides non-coding transcripts such as *XIST*, XCI involves chromatin modifiers and factors of nuclear organization (Chow and Heard, 2009). Together these lead to a changed chromatin structure and the spatial reorganization of the then silenced X chromosome. *XIST* itself is regulated by CpG-sites of the promoter region which, if methylated, repress transcription on the active X chromosome, and are, in contrast, unmethylated and transcriptionally active on the inactive X.

Whereas it is quite obvious that the PARs are not inactivated to achieve the same gene-dosage in both sexes, things get more complicated as also single genes and whole regions of the 'inactive' X chromosome-specific sections are actually not silenced already in females (Sudbrak *et al.*, 2001). By evaluating the expression of X-linked genes, it was found that ~30% of the genes on Xp and in contrast <3% of the genes on Xq may escape inactivation. In total, ~5% of X-linked genes escape inactivation and an additional 10% show a variable pattern of XCI (Carrel *et al.*, 1999; Carrel and Willard, 2005).

The KS phenotype may therefore reflect either two active copies of these strictly X-linked genes or three active copies of X–Y homologous genes (from the PARs) because of gene-dosage (more active copies presumably leading to a higher gene expression). Admittedly, for the former, it is necessary to postulate that somatic cells in 47,XXY males inactivate the supernumerary X in the same manner as those in females. This assumption has gained support lately, when Monkhorst *et al.* (2008, 2009) showed that XCI is related to the X to autosomal ratio (which is the same in 47,XXY males as in 46,XX females), at least in polyploid mouse embryonic stem cells. Additional studies investigating XCI specifically in KS are presented below.

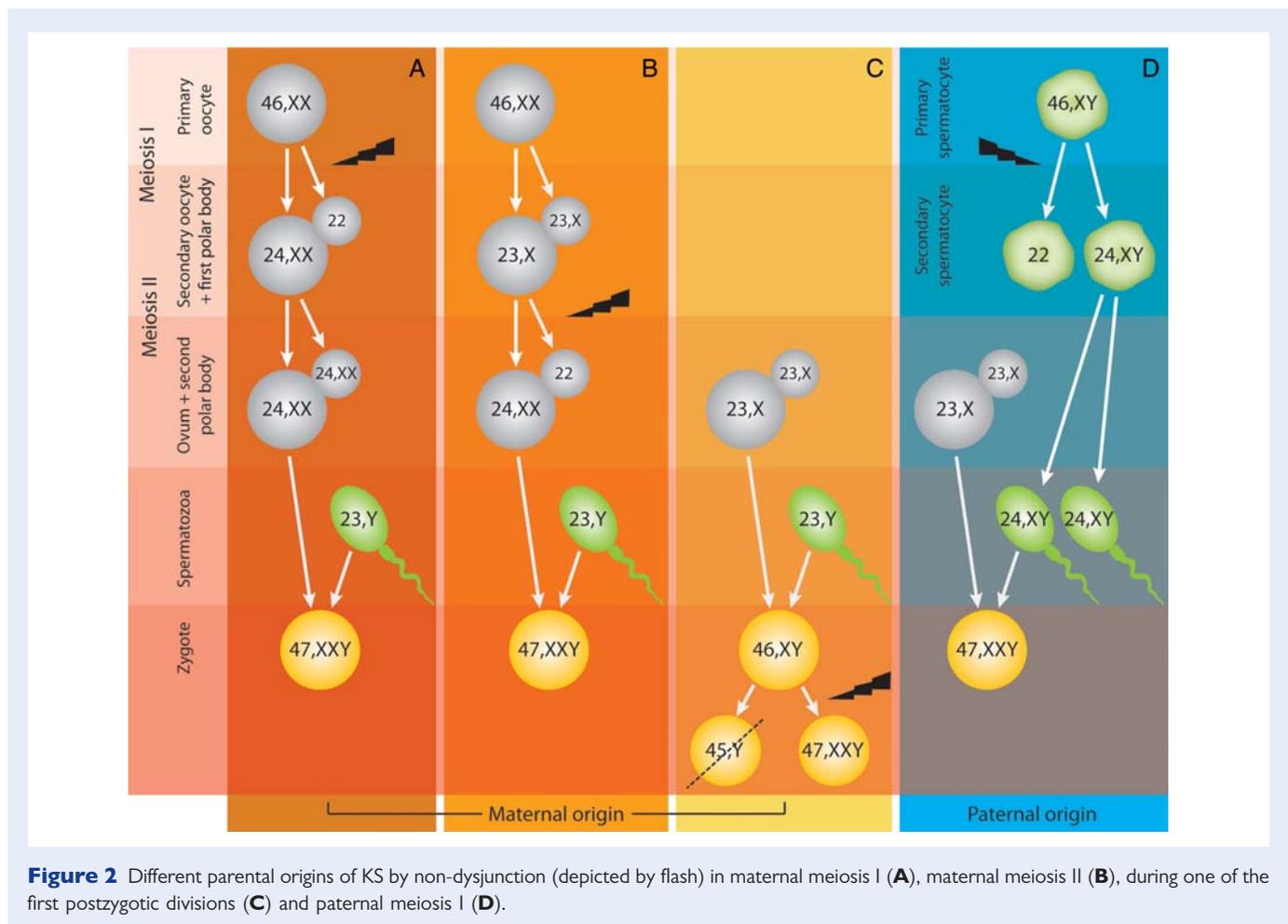
## Origin of Klinefelter's syndrome

In opposition to autosomal trisomies, which only in a minority of ~10% are paternally derived, the supernumerary X in half of KS cases originates from paternal non-disjunction (Thomas and Hassold, 2003). Although maternal XXY can be caused by non-disjunction during the first and second meiotic divisions or during early postzygotic mitotic divisions in the developing zygote, XXY of paternal origin can arise only by meiosis I errors as paternal non-disjunction during meiosis II leads to XXX or XYY zygotes (Fig. 2, Lanfranco *et al.*, 2004). An association of the frequency of KS with increasing maternal age at conception has been reported in

accordance with other (primarily 13, 18, 21) chromosome trisomies (Hook, 1981), and a study by Harvey *et al.* (1990) could attribute this increase of KS to maternal meiosis I errors. In contrast, an association of paternally derived XXY with the father's age remains debatable among some confirming studies (Carothers and Filippi, 1988; Lorda-Sanchez *et al.*, 1992) and some contradictory reports (Jacobs *et al.*, 1988; Harvey *et al.*, 1990; MacDonald *et al.*, 1994). Evidence for such an association would come from the finding that sperm aneuploidies increase with age (Eskenazi *et al.*, 2002; Arnedo *et al.*, 2006), which is, however, also objected by others (Luetjens *et al.*, 2002; Martin, 2008). The reported increase in KS prevalence over the last decades was attributable only to paternal origin and hypothesized to be caused by environmental factors interfering with paternal meiosis I (Morris *et al.*, 2008). As Herlihy and Halliday (2008) pointed out, a more obvious reason might well be an overall increasing paternal age, but this needs to be tested by detailed analyses of larger cohorts.

## Pathophysiology and genotype/phenotype

Rough estimates of the pathophysiology of KS may be derived from comparing the phenotype associated with the 'classic' 47,XXY constitution with other sex chromosome aneuploidies. Among others, the



correlation with the phenotype is more firmly established in higher order sex chromosome aneuploidies (48,XXXY etc.) than in KS: the clinical picture progressively deviates from normal as the number of X chromosomes increases and the frequency of almost any somatic anomaly is higher compared with 47,XXY (Visootsak *et al.*, 2001). XXXY and XXXXY males present with characteristic facial and skeletal malformations, intrauterine growth retardation and psychomotor retardation (Linden *et al.*, 1995; Simsek *et al.*, 2009). Another rare, closely related sex chromosomal aneuploidy, the 48,XXYY syndrome, with a prevalence of  $\sim 1/18\,000$  to  $1/40\,000$  displays physiological patterns similar to KS such as tall stature, hypergonadotropic hypogonadism and infertility, but differently from KS and like the other higher order aneuploidies is associated with significantly more severe neurodevelopmental and psychological features (Tartaglia *et al.*, 2008). Similar to KS, these men exhibit a remarkable phenotypic variation which, like in KS, might be influenced by DNA methylation effects and/or the (CAG) $_n$  repeat polymorphism of the androgen receptor (both discussed later). On the basis of reports of KS patients with X isochromosomes (a chromosome that has lost one of its arms and replaced it with a copy of the other arm), the long arm of the X chromosome (Xq) seems to primarily contribute to the KS phenotype (Arps *et al.*, 1996; Höckner *et al.*, 2008). Interestingly, the phenotype closely resembles that of 47,XXY KS, but with the important exception of tall stature which thus should be related to Xp.

As described above, in 47,XXY men, the supernumerary X chromosome is inherited from the mother and the father in  $\sim 50\%$ , respectively (Thomas and Hassold, 2003), and may affect the phenotype by differential expression of paternal versus maternal alleles, i.e. imprinting (Iitsuka *et al.*, 2001). Apart from that, maternal

non-disjunction during meiosis I leads to uniparental heterodisomy (two different X chromosomes from the same parent, in this case the mother), while an error during meiosis II results in uniparental isodisomy (duplicate of one maternal X chromosome in the child). If the father contributes the extra X, the child will bear two different X chromosomes of different paternal origin (Fig. 1). To date, six studies analyzed parental origin with respect to the KS phenotype with inconsistent results (Table I): four studies investigated a wide range of features from anthropometric measures (including penile length and testicular volume), hormones, psychotic symptoms, to cognitive and motor development and did not find any differences between KS patients carrying a paternal compared with a maternal extra X (Zinn *et al.*, 2005; Ross *et al.*, 2006, 2008; Zeger *et al.*, 2008). On the other hand, in their study of 61 KS men, Stemkens *et al.* (2006) demonstrated a higher incidence of developmental problems in speech/language (88% versus 59%) and motor impairment (77% versus 46%) when the supernumerary X chromosome was paternally inherited. In addition, they found all anthropometric measures related to body size greater in the paternal X group, although only head circumference, sitting height and penile length reached statistical significance. Body height was borderline significantly higher ( $P = 0.05$ ). In concordance with an influence of the parental origin, Wikström *et al.* (2006) described a later onset of puberty indicated by clinical markers (Tanner stage) and hormone measurements in the paternal X group, albeit in a small group of 14 boys. This is to date also the only study analyzing hetero- versus isodisomy and the authors did not find an influence on the phenotype.

The human androgen receptor (AR, previously also HUMARA, located in Xq11.2–q12) is of double interest concerning genotype/phenotype correlations in KS. The AR contains a highly polymorphic

**Table I** Summary of studies reporting parental origin of the supernumerary X chromosome, X inactivation, androgen receptor (AR) (CAG) $_n$  repeat length, or combinations associated with KS phenotype.

Study	KS subjects (n: age)	Outcome measures	Genetic analyses	Results
Zitzmann <i>et al.</i> (2004)	77: 18–65 years	Anthropo- and sociometrical data, features of hypogonadism (gynecomastia, etc.), hormones	X inactivation, AR (CAG) $_n$	AR (CAG) $_n$ positively correlated with body height and predictive for gynecomastia and smaller testes; AR (CAG) $_n$ inversely correlated with bone density, stable partnership and higher education
Zinn <i>et al.</i> (2005)	35: 0.1–39 years	Anthropometric measurements including penile length and testicular volume	Parental origin, X inactivation, AR (CAG) $_n$	AR (CAG) $_n$ inversely correlated with penile length
Stemkens <i>et al.</i> (2006)	61: 2–56 years	Anthropometric and psychomotor development, IQ	Paternal origin	Impaired speech and motor development problems more often in paternal X cases
Ross <i>et al.</i> (2006)	11: 19–54 years	Psychotic symptoms	Parental origin, X inactivation	No association
Wikström <i>et al.</i> (2006)	14: 10–13.9 years	Pubertal development, growth, testicular volume, hormones	Parental origin, iso/heterodisomy, X inactivation, AR (CAG) $_n$	Paternal origin of X chromosome associated with later onset of puberty; longer AR (CAG) $_n$ with later reactivation of pituitary-testicular axis
Ross <i>et al.</i> (2008)	50: 4.1–17.8 years	Cognitive and motor development	Parental origin, X inactivation, AR (CAG) $_n$	No associations
Zeger <i>et al.</i> (2008)	55: 2.0–14.6	Anthropometric measurements including penile length and testicular volume; hormones	Parental origin	No association

trinucleotide repeat (CAG)<sub>n</sub> in exon I (Choong and Wilson, 1998) with the normal length varying between 9 and 36/37 repeats (Zitzmann and Nieschlag, 2003); expanded repeats are associated with the neurological disorder of X-linked spinobulbar muscular atrophy (La Spada *et al.*, 1991). The (CAG)<sub>n</sub> repeat is correlated with physiological androgen effects in healthy men and probably has pharmacogenetic implications as well, because testosterone treatment effects seem to be modulated by its number (reviewed in Zitzmann, 2009). On the contrary, a long sought association of the (CAG)<sub>n</sub> with male infertility remains elusive (Davis-Dao *et al.*, 2007; Tüttelmann *et al.*, 2007). Since the AR contains two methylation-sensitive *HpaII* restriction sites close to the (CAG)<sub>n</sub> repeat, a comparison of PCR products obtained before (both alleles) and after (only inactive, i.e. methylated allele) digestion can also be used to detect XCI (Allen *et al.*, 1992). In subjects with two X chromosomes (females and KS alike), an estimation of the biological activity of the AR is not as straightforward as in 46,XY males with just one copy, but should depend on the (CAG)<sub>n</sub> length corrected for the XCI ratio. Therefore, the calculation of a so-called 'X-weighted mean' taking both into account has been introduced and correlations with clinical features have been described (Hickey *et al.*, 2002), including KS (Table I). We found a positive correlation between (CAG)<sub>n</sub> length and body height and an inverse relation with bone density and arm span to body height ratio in a large study of 77 KS men. In addition, the presence of long (CAG)<sub>n</sub> had predictive power for having gynecomastia and smaller testes, whereas short (CAG)<sub>n</sub> were associated with a stable partnership and professions requiring higher education; clinical measures (LH suppression, prostate growth, hemoglobin concentration) under testosterone substitution were also correlated (Zitzmann *et al.*, 2004). Subsequently, Zinn *et al.* (2005) described an inverse relationship of (CAG)<sub>n</sub> and penile length and Wikström *et al.* (2006) reported an association of longer (CAG)<sub>n</sub> with a later reactivation of the pituitary–gonadal axis in KS boys. On the contrary, Ross *et al.* (2008) could not find an influence of (CAG)<sub>n</sub> length on

cognitive and motor development in a quite large study of 50 KS boys. The common notion that AR activity is inversely correlated to (CAG)<sub>n</sub> repeat length derived from *in vitro* and *in vivo* studies has recently been challenged by a new *in vitro* study which might possibly also explain the discrepant findings *in vivo* (Nenonen *et al.*, 2010).

Assuming a random XCI, the ratio of activation/inactivation at any X-chromosomal allele outside the PARs would be expected to be 50%. Conversely, while analyzing XCI in KS, a skewed inactivation, usually defined as above 80% activation/inactivation, of one allele was detected in a variable percentage of cases. When all studies published so far are reviewed, the percentage of skewed XCI ranges from below 10 to over 40 (Table II). Furthermore, we found a preferential inactivation of the shorter allele (Zitzmann *et al.*, 2004), which would magnify the impact of the (CAG)<sub>n</sub> length, but this has not been replicated so far. In contrast, Suzuki *et al.* (2001) found a generally preferred inactivation of the longer allele (but only analyzed seven men), whereas the two successive studies found no preferential XCI at all (Zinn *et al.*, 2005; Wikström *et al.*, 2006).

Further studies investigated XCI of other genes than the AR but essentially remain single reports. Ross *et al.* (2006) described that in KS men, *PCDH11X/Y* is unmethylated (three active copies) and escapes XCI in contrast to *SYBL1*. Both genes are expressed in the brain and were hypothesized to play a role in the cognitive phenotype of KS. *PCDH11X/Y* is located in the human XY non-PAR homology region in Xq21.3 and *SYBL1* in PAR2 (Helena Mangs and Morris, 2007; Wilson *et al.*, 2007). By using a whole-genome expression array, Vawter *et al.* (2007) found differential expression of 129 genes comparing 11 KS and 6 XY males with X-chromosomal genes being overrepresented (14 of 129). The authors also describe correlations of the differentially expressed genes with measures of verbal cognition, but the sample size is probably too small to draw definite conclusions. In a different approach using pyrosequencing, Chung *et al.* (2006) investigate inactivation of X-chromosomal genes. They identified 14 genes escaping XCI, of which 7 show a profile

**Table II Summary of studies analyzing X inactivation in KS patients.**

Study	Subjects (n)	Locus analyzed	X inactivation*
litsuka <i>et al.</i> (2001)	14 KS	AR (CAG) <sub>n</sub>	21% (3) skewed
Suzuki <i>et al.</i> (2001)	7 KS	AR (CAG) <sub>n</sub>	43% (3) skewed, longer allele preferred overall
Zitzmann <i>et al.</i> (2004)	46 KS	AR (CAG) <sub>n</sub>	11% (5) skewed, shorter allele preferred
Zinn <i>et al.</i> (2005)	22 KS	AR (CAG) <sub>n</sub>	9% (2) skewed, no preferential allele
Wikström <i>et al.</i> (2006)	6 KS	AR (CAG) <sub>n</sub>	33% (2) skewed, no preferential allele
Ross <i>et al.</i> (2008)	26 KS	AR (CAG) <sub>n</sub>	8% (2) skewed
Ross <i>et al.</i> (2006)	11 KS	<i>SYBL1</i> **	2 methylated, 1 unmethylated, comparable to females
		<i>PCDH11X/Y</i> **	All 3 unmethylated (escapes inactivation)
Chung <i>et al.</i> (2006)	5 KS, 5 XX	14 genes	7 escape inactivation, comparable to females; other 7 genes without clear result
Poplinski <i>et al.</i> (2010)	10 KS, XY, XX each	<i>XIST</i> , <i>PGK1</i> <i>SHOX</i> <i>FTHL17</i>	50% methylated as in females Low, comparable to females High, comparable to females

\*>80% methylation, skewed inactivation.

\*\*Gene from the pseudoautosomal region, three gene copies in KS patients.

comparable to females, whereas the results of the other 7 genes did not suffice for definite conclusions. Recently, we could furthermore demonstrate a comparable *XIST* promoter methylation of 50% in KS comparable with that in females. Low methylation (below 10%) of *SHOX* (from PARI), ~50% methylation *PGKI* (indicator of XCI) and high methylation (above 90%) of *FTHL17* were also similar in 47,XXY and 46,XX (Poplinski *et al.*, 2010).

Summarizing, the role of the AR (CAG)<sub>n</sub> repeat polymorphism has been studied quite extensively in KS boys and men, but without reaching a uniform picture. Neither the number of repeats nor the XCI pattern is uniformly associated with aspects of the KS phenotype, which might primarily be due to the wide range of features under investigation (e.g. psychological and anthropometric measures, hormones), but also in part to the heterogeneous study protocols of highly variable sample sizes and age groups. Whether XCI is skewed more often in KS than in females or the noted percentages of skewed XCI are just the extremes of a Gaussian distribution remains to be determined. In addition, the question arises whether aging affects (skewed) XCI in KS as has been postulated in women (Sharp *et al.*, 2000) which is currently a topic of intensive debate (Swierczek *et al.*, 2008; Busque *et al.*, 2009). Overall, the collected data, albeit sparse and regularly limited to single studies, support that (i) XCI in KS follows the same pattern as in females and (ii) therefore XCI escapees in females probably also escape inactivation in KS, then possibly being overexpressed and involved in the phenotype.

Further knowledge may be gained by comparing KS with other sex chromosomal aneuploidies, especially 45,X (Turner's syndrome) females and 46,XX males. Turner's syndrome is characterized by a completely or in part missing second X chromosome and approximately affects 1 in 2000–2500 live female births (Nielsen and Wohler, 1990; Stochholm *et al.*, 2006). Over 90% of patients exhibit the common features of short stature and premature ovarian failure (Bondy, 2009). The XX male syndrome on the other hand is rare, occurring approximately in 1 in 20 000 newborn males. The phenotype had not been well defined, but was recently described in detail from our collection of 11 affected men (Vorona *et al.*, 2007). In agreement with others (Aksglaede *et al.*, 2008), we found 46,XX males to be of significantly shorter stature than healthy as well as KS men while otherwise quite similar to KS. Concerning the KS phenotype, both entities are interesting with respect to the explanation of increased body height in XXY. Short stature in Turner's syndrome is caused by haploinsufficiency for the pseudoautosomal gene *SHOX* encoding a transcription factor expressed in the developing skeleton and implicated in various skeletal anomalies seen in 45,X. Consistent with these data, 46,XX males reach a mean body height lower than control males but comparable to healthy females. The *SHOX* gene's role in short stature is firmly established starting from Turner syndrome expanding to idiopathic short stature (Ellison *et al.*, 1997; Rao *et al.*, 1997; Blaschke and Rappold, 2006). Fittingly, *SHOX* is overall non-methylated in 46,XX males and females (Poplinski *et al.*, 2010), and the abnormal growth patterns cannot be explained by different serum levels of IGF-I and IGFBP-3 (exerting growth hormone effects). Furthermore, KS boys exhibit an accelerated growth already from an age of 6 years onward, when an effect of sex hormones is highly unlikely (Aksglaede *et al.*, 2008). An effect of *SHOX* overdosage was reported by several authors in females with varying supernumerary X chromosome constitutions (Adamson *et al.*, 2002;

Kanaka-Gantenbein *et al.*, 2004; Alvarez-Vazquez *et al.*, 2006; Nishi *et al.*, 2008). Consequently, the tall stature in KS may not be mainly due to hypogonadism as previously thought, i.e. lower testosterone/estrodial levels not stopping long-bone growth by inducing epiphyseal growth plate fusion. On the contrary, increased body height may well be caused by excessive expression of growth-related genes with *SHOX* as the leading candidate as 47,XXY carry three copies. Therefore, *SHOX* can be considered an example of a gene-dosage effect of a pseudoautosomal gene in KS.

Another example arises from investigations of autoimmune disease, which usually show a marked predominance in women. For systemic lupus erythematosus (SLE), Scofield *et al.* (2008) recently described a high prevalence of KS of 1 in 43 men in their cohort of male SLE patients equaling an ~14-fold increase in comparison to the population frequency. Consequently, the risk for SLE in KS men is comparable to that in 46,XX females and ~14-fold higher than in 46,XY men. Interestingly, only one of these five men had been diagnosed with KS before. Moreover, an underrepresentation of females with Turner's syndrome may exist, but cannot be reliably determined from the data available. Hence, an involvement of gene-dosage effects and/or XCI in the pathogenesis of SLE and probably other autoimmune diseases is likely (Selmi, 2008; Sawalha *et al.*, 2009).

## Fertility

The X chromosome exhibits genes (99 out of 1098) specifically expressed in the testis (Ross *et al.*, 2005). Thus, it is not surprising that also the fertility status of XXY patients is affected and highly variable (Aksglaede *et al.*, 2006). With the advent of microdissection (microsurgical) testicular sperm extraction, the chances to retrieve spermatozoa in KS patients are reported to range between 30% and 70% (Schiff *et al.*, 2005; Koga *et al.*, 2007; Yarali *et al.*, 2009) and the possibility for KS men to become fathers utilizing *in vitro* fertilization with intracytoplasmic sperm injection (ICSI) arises. Concurrently, the main question of how spermatogenesis is disturbed in KS or, put the other way around, how it still works, becomes of increasing importance with respect to the risk for the offspring of, for example, chromosomal aneuploidy. The degeneration of germ cells in KS may in principal be caused by the supernumerary X itself preventing the completion of meiosis, or, on the other hand, a disturbed testicular environment involving somatic Sertoli and Leydig cells (Aksglaede *et al.*, 2006), which currently remains unresolved. On the contrary, recent findings may shed light on the long debated question whether 47,XXY spermatogonia are able to complete meiosis or, in contrast, some spermatogonia lose the supernumerary X chromosome becoming normal 46,XY cells and then proceed through meiosis. While others previously postulated the completion of meiosis of 47,XXY spermatogonia supported by indirect clues (Foresta *et al.*, 1999), Sciurano *et al.* (2009) nicely showed by FISH analyses that all meiotic spermatocytes were euploid 46,XY. In addition, the frequency of sperm sex chromosome aneuploidies would be expected to be as high as 50% if 47,XXY spermatogonia were meiotically competent, while this recent work further fits an at most slightly elevated risk in KS men (reviewed in Hall *et al.*, 2006; Martin, 2008). Concordantly, the outcome of children of KS fathers is overall reassuring, albeit a minor, but significant, increase of incidence of KS boys and, surprisingly, also of autosomal aberrations

has been reported (Staessen *et al.*, 2003). Because the analyzed number of pregnancies from KS fathers is still low with around 200 cases, all of which, of course, achieved by ICSI which in itself bears a slightly higher risk for chromosomal aberrations, final conclusions cannot be drawn.

A topic gaining interest is fertility preservation by cryopreservation of immature testicular tissue in prepubertal boys undergoing gonadotoxic therapies because of malignant disease. This would also be an interesting option for KS patients, since degeneration of seminiferous tubules in KS seems to accelerate with puberty (Wikström and Dunkel, 2008). Testicular biopsies obtained earlier and then permanently stored (established) could be used to derive gametes for fertilization in the future by *in vitro* maturation (including meiosis), which at present, however, remains entirely experimental (Wyns *et al.*, 2010). The prerequisite for such a procedure would be the earlier diagnosis of KS boys, though, which could be achieved by introducing the new and inexpensive screening methods like qPCR (presented earlier).

One miscellaneous issue concerning KS and fertility is the analysis of microdeletions of the Y chromosome, i.e. AZF (azoospermia factor) deletions. Mitra *et al.* (2006) reported a surprisingly high incidence of AZFa and AZFb deletions in 4 out of 14 KS patients, which was not confirmed by screening of large numbers (>200) of KS patients from our patient cohort (Simoni *et al.*, 2008) and others (Choe *et al.*, 2007). Nevertheless, another study was carried out with an amazingly high percentage of six out of nine KS patients supposed to bear an AZF deletion (Hadjkacem-Loukil *et al.*, 2009). Both studies reporting this high microdeletion prevalence have to be questioned because the deletions presented were rather unconventional, mostly involving only isolated markers of the AZFa, AZFb and/or AZFc regions. Since these deletions were not confirmed with an independent method such as Southern blotting, they should probably be regarded as methodological artifacts. Indeed, based on the much larger cohorts of patients, it was confirmed that deletions of the Y chromosome do not occur in patients with KS.

## Genetic counseling

With respect to KS, genetic counseling is necessary in the situations of a prenatal diagnosis of KS and for couples planning ICSI as in all cases of male-factor infertility. Considering the high variability of the phenotype, in a large proportion with a benign clinical picture not even diagnosed throughout life, a rate of 70% induced abortion after prenatal diagnosis of KS (Bojesen *et al.*, 2003; Hamamy and Dahoun, 2004) seems high. Meschede *et al.* (1998) reported a markedly lower rate of pregnancy termination which may depend on cultural differences in parental perception of sex chromosomal polysomies but probably also on characteristics of genetic counseling at our institution. Health professionals providing genetic counseling influence the parents' decision against or toward pregnancy termination with the pregnancy more likely to continue if the counseling is given by a specialized geneticist (Hall *et al.*, 2001; Marteau *et al.*, 2002). When ICSI is planned, the genetic risks resulting from this procedure should be discussed with each couple. The above-mentioned, probably slightly increased risks for autosomal as well as sex chromosomal aberrations arising by the 47,XXY constitution of the father and to some extent implicated through ICSI itself need to be discussed. The potential benefits and risks of preimplantation or prenatal

diagnosis (by chorionic villous sampling or amniocentesis) genetic diagnosis should be considered depending on the technical and legal availability. Summing up, genetic counseling is recommended in all cases of a new diagnosis of KS whether pre- or post-natally and in any case of couples undergoing ICSI.

## Conclusions

The frequency and variability of KS make it the most common as well as heavily underdiagnosed chromosome aneuploidy in men. The currently available knowledge provides hints to the pathophysiology and genotype/phenotype correlations of the supernumerary X chromosome. Gene-dosage effects, of which *SHOX* related to the tall stature in KS is a leading example, in combination with (escapes from) XCI, are most likely constituting the phenotype. Lessons can also be learned from comparisons with normal males and especially females as well as other sex chromosomal aneuploidies. The recently described higher incidence of autoimmune diseases in KS implicates a need to intensify screening also in these patient groups and not only focus on male infertility and the 'classic' phenotype.

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