Received: 7 February 2020 | Revised: 6 May 2020 | Accepted: 9 May 2020

DOI: 10.1002/ajmg.c.31802

RESEARCH REVIEW

AMERICAN JOURNAL OF medical genetics PART C Seminars in Medical Genetics

WILEY

Epigenetics and genomics in Klinefelter syndrome

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Funding information
Aase og Ejnar Danielsens Fond; Augustinus Fonden; Foundation of 17-12-1981; Health Research Fund of Central Region Denmark; Lundbeckfonden; Novo Nordisk Fonden

Abstract

Since the first description of Klinefelter syndrome (KS) was published in 1942 in *The Journal of Clinical Endocrinology*, large inter-individual variability in the phenotypic presentation has been demonstrated. However, our understanding of the global impact of the additional X chromosome on the genome remains an enigma. Evidence from the existing literature of KS indicates that not just one single genetic mechanism can explain the phenotype and the variable expressivity, but several mechanisms may be at play concurrently. In this review, we describe different genetic mechanisms and recent advances in the understanding of the genome, epigenome, and transcriptome of KS and the link to the phenotype and clinical heterogeneity. Future studies are needed to unite clinical data, genomic data, and basic research attempting to understand the genetics behind KS. Unraveling the genetics of KS will be of clinical relevance as it may enable the use of polygenic risk scores to predict future disease susceptibility and enable clinical risk stratification of KS patients in the future.

KEYWORDS

epigenetics, genomics, genotype, Klinefelter syndrome, phenotype, sex chromosomes

1 | INTRODUCTION

Klinefelter syndrome (KS) is the most common sex chromosome disorder as well as one of the most frequent genetic abnormalities. The syndrome is due to the presence of a supernumerary X chromosome in males and occurs with an estimated prevalence range from 85 to 250 per 100.000 liveborn males (Gravholt et al., 2018). Since the first description of Klinefelter syndrome was published in 1942 in *The Journal of Clinical Endocrinology* (Klinefelter, Reifenstein, & Albright, 1942) large inter-individual variability in the phenotypic presentation and associated co-morbidities has been demonstrated. The phenotype may vary ranging from men with KS exhibiting classical traits including hypergonadotropic hypogonadism, infertility and neurocognitive deficits in addition to severe comorbidities such as psychiatric disorders, obesity, diabetes, osteoporosis, and autoimmune disorders to men with KS having almost no traits or comorbidities except from small testes and infertility (Gravholt et al., 2018). The spectrum of co-morbidity spans from diseases of the circulatory and respiratory system over endocrine, metabolic, and bone disorders to diseases of the digestive system and genitourinary system in addition to diseases of the nervous system. Evidence suggests that almost 50% suffer from metabolic syndrome and abdominal adiposity, type 2 diabetes is seen in 10–39%, osteoporosis is seen in 5–10% with approximately 40% having osteopenia and psychiatric disturbances is seen in around 30–50% of men with KS (Bojesen, Juul, Birkebaek, & Gravholt, 2006; (Bojesen et al., 2006; Chang et al., 2019; Swerdlow, Higgins, Schoemaker, Wright, & Jacobs, 2005). However, our understanding of the global impact of the extra X chromosome still remains an enigma. Several genetic mechanisms and hypotheses have been proposed and investigated in an attempt to explain the clinical phenotype and the within-syndrome variability. However, the molecular basis for the phenotypic presentation, the increased susceptibility to a broad range of disorders, and how the increased X chromosome dosage predisposes and mediates its effect on the genome are still poorly understood. In this article, we will describe recent advances in the understanding of the genome, epigenome, and transcriptome of KS.

2 | KARYOTYPE AND MOSAICISM

The karyotypes seen in men with KS encompass 47,XXY, accounting for 85–90% of cases, the mosaic karyotype 46,XY/47,XXY, seen in 6–7% of cases, in addition to rare karyotypes including 46,XX/47,XXY (Bojesen, Juul, & Gravholt, 2003; Nielsen & Wohlert, 1990).

Mosaicism is considered an important factor contributing to clinical heterogeneity in disorders (Youssoufian & Pyeritz, 2002). Mosaicism is a common phenomenon in Turner syndrome (TS; 45,X) (Gravholt, Juul, Naeraa, & Hansen, 1996) and mosaic karyotype in these women are associated with a milder phenotype with reduced severity of congenital heart diseases and enhanced fertility compared to nonmosaic TS women (Bernard et al., 2016; Cameron-Pimblett, La, King, Davies, & Conway, 2017; El-Mansoury et al., 2007). Evidence suggest that mosaicism may be more frequent in KS than previously thought based on conventional cytogenetic analysis of blood samples, as mosaicism may vary between tissue types and be more frequent in other cell types than lymphocytes (Garcia-Quevedo et al., 2011). Here, the authors reported the highest degree of mosaicism in Sertoli cells (42.3% ± 11.1%) and the lowest degree of mosaicism in lymphocytes 4.8% ± 2.5%, whereas the degree of mosaicism was 21.9 ± 10.9% in buccal mucosa. It is likely that mosaicism within men with KS may play a to date overlooked, essential role in the diversity of the clinical manifestation seen in men with KS. Further studies are needed to elucidate a possible impact of mosaicism on the phenotype.

Usually, men with KS and a 46,XY/47,XXY mosaic karyotype, based on conventional cytogenetic analysis on blood samples, have been described as presenting with a milder phenotype with reduced severity of several phenotypic traits compared to nonmosaic KS males (Lanfranco, Kamischke, Zitzmann, & Nieschlag, 2004; Paduch, Fine, Bolyakov, & Kiper, 2008). This is in agreement with an increased age at diagnosis compared to the mean age at diagnosis in men with KS (37 years vs. 27 years) (Gravholt et al., 2018; Samplaski et al., 2014). Samplaski et al. reported larger mean testicular volume, higher mean total sperm count, lower baseline luteinizing hormone and estradiol levels in men with KS and a 46,XY/47,XXY mosaic karyotype (n = 6) compared to nonmosaic KS males (Samplaski et al., 2014), but the results need to be replicated, because of the few individuals examined in that study. No data on morbidity in relation to karyotype have been presented, however mortality was reported to be equal between men with KS and a 46,XY/47,XXY mosaic karyotype and nonmosaic KS males (Bojesen, Juul, Birkebaek, & Gravholt, 2004; Chang et al., 2019). Regarding socioeconomic status (e.g., cohabitation, marriage, education, income, and retirement), men with KS and a 46,XY/47,XXX mosaic karyotype resemble the profile seen in nonmosaic KS males (Bojesen, Stochholm, Juul, & Gravholt, 2011).

The prevalence of men with KS and a 46,XX/47,XXY mosaic karyotype is very low, with only few case reports published (Ford, Polani, Brigs, & Bishop, 1959; Hecht, Antonius, McGuire, & Hale, 1966; Matsuki, Sasagawa, Kakizaki, Suzuki, & Nakada, 1999; Mohd Nor & Jalaludin, 2016; Nowakowa, Lenz, Bergman, & Reiitalu, 1960; Song, Lee, Jin, & Kim, 2014; Velissariou et al., 2006), thus our knowledge about the phenotypic presentation of these men with KS is limited. The spectrum of clinical manifestations described in case reports include hypergonadotropic hypogonadism, cryptorchidism, small testis, overweight, eunuchoid body habitus and mild gynecomastia, all of which are phenotypic traits characteristic of men with KS. However, the 46,XX/47,XXY mosaic karyotype may also result in disorder of sex development (Bergmann, Schleicher, Bocker, & Nieschlag, 1989; Isguven et al., 2005; Kanaka-Gantenbein et al., 2007; Nihoul-Fekete, Lortat-Jacob, Cachin, & Josso, 1984; Ozsu et al., 2013; Perez-Palacios et al., 1981) or a normal female karyotype (Hamlett, Timson, & Harris, 1970).

3 | PARENTAL ORIGIN AND X CHROMOSOME INACTIVATION

Through decades, several genetic mechanisms linked to the X chromosome have been hypothesized to hold the key for understanding the phenotype. In 1989, Jacobs et al. (Jacobs, Hassold, Harvey, & May, 1989) proposed that imprinted genes on the X chromosome might be involved in the phenotypic variability seen in KS, as 50% of cases is due to nondisjunction in the paternal meiotic division (Jacobs et al., 1988; Thomas & Hassold, 2003) and the other 50% of KS cases is due to nondisjunction in maternal meiosis or during post zygotic mitosis (Jacobs et al., 1988; Thomas & Hassold, 2003). However, the role of imprinted gene on phenotypic traits in KS is still highly speculative, as no imprinted genes on the X chromosome have been identified. Yet, autistic and schizotypical traits, motor and language function, onset of puberty and a few anthropometric measurements have been attributed to parental origin of the X chromosome (Bruining et al., 2010; Chang et al., 2015; Stemkens et al., 2006; Wikstrom, Painter, Raivio, Aittomaki, & Dunkel, 2006); however evidence is sparse as these results are based on few studies and because other studies have not been able to find evidence of an parent-of-origin effect (Ross et al., 2008; Skakkebaek et al., 2014; Zeger et al., 2008; Zinn et al., 2005).

In addition, skewed X chromosome inactivation (defined as above 80% preferential inactivation of one of the X-chromosomes) has been suggested to account for some of the clinical heterogeneity seen among KS. In cells with two X chromosomes, one of the X chromosomes is subjected to X chromosome inactivation early in embryogenesis (Lyon, 1961). In this process, the majority of X chromosomal genes on the X chromosomes are silenced. This process is accompanied by X chromosome upregulation of the active chromosome to equalize gene dosage relative to autosomes. The inactivation normally occurs randomly resulting in an equivalent fraction of inactivated paternal and maternal X chromosomes. Skewed X chromosome inactivation could result in either a silencing of maternally or paternally imprinted genes, and Skuse et al. have proposed that some X-linked genes may be imprinted (Skuse et al., 1997). In KS, skewed X chromosome inactivation has been reported in up to 43% of cases (Tuttelmann & Gromoll, 2010). The current literature, however, have not found evidence that KS patients with skewed X chromosome demonstrate a different phenotype compared to men with KS with a random X-zinactivation (Bojesen, Hertz, & Gravholt, 2011; Chang et al., 2015; Ross et al., 2006; Ross et al., 2008; Skakkebaek et al., 2014; Zinn et al., 2005; Zitzmann, Depenbusch, Gromoll, & Nieschlag, 2004). Suffice to say, in order to establish a firm role for both parental origin of the second X chromosome and skewed X chromosome inactivation, there is a need for much larger unbiased studies.

4 | ANDROGEN RECEPTOR

The androgen receptor (AR) gene has drawn attention in KS research, as hypergonadotropic hypogonadism is a hallmark in men with KS. This gene mediates the peripheral effects of testosterone, is located on the X chromosome (Xq11-12), and is expressed in virtually all tissues (Davey & Grossmann, 2016). The AR gene contains a polymorphic polyglutamine (CAG) repeat sequence in exon 1 and the activity of the androgen receptor have been demonstrated to correlate negatively to the length of this CAG repeat (Chamberlain, Driver, & Miesfeld, 1994). It has been proposed that the repeat length may be related to the phenotypic variability seen in KS. The CAG repeat length has been demonstrated to exert an impact on anthropometric measures such as height (Bojesen, Hertz, & Gravholt, 2011; Bojesen, Stochholm, et al., 2011; Zitzmann et al., 2004), arm span (Bojesen, Hertz, & Gravholt, 2011; Bojesen, Stochholm, et al., 2011; Chang et al., 2015; Zitzmann et al., 2004), and arm/leg length (Chang et al., 2015), in accordance with the inverse correlation between CAG repeat length and later onset of pubertal reactivation of the HPG-axis (Wikstrom et al., 2004). On the contrary, no link seems to exist between the CAG repeat length and neurocognitive functions (Ross et al., 2017; Skakkebaek et al., 2014), whereas the relations between phenotypic traits such as bi-testicular volume, penile length, gynecomastia, lipid metabolism, bone parameters and the CAG repeat length, are more uncertain as results diverge (Bojesen, Hertz, & Gravholt, 2011; Bojesen, Stochholm, et al., 2011; Chang et al., 2015; Zinn et al., 2005; Zitzmann et al., 2004).

Allelic variants of the AR gene have been associated with phenotypic abnormalities in individuals with 46,XY, including development of androgen insensitivity syndrome. Valente et al. evaluated whether allelic variants of the AR gene could account for some of the phenotypic variability seen in spermatogenesis, sex hormones levels and metabolic parameters in men with KS (Valente et al., 2017). The authors showed that 5.9% of men with KS carry an allelic variant of the AR gene, of which 66% (4/6) had previously been associated with different levels of androgen insensitivity, whereas 33% (2/6) were novel variants. However, no differences in clinical parameters between carriers and noncarriers were seen. Larger phenotype–genotype studies are needed to further determine whether allelic variants in the AR gene affect the phenotype in men with KS.

5 | GENETIC VARIANTS OF AUTOSOMAL GENES

Genetics variants underlie differences in our susceptibility to a wide range of diseases. The extra X-chromosome could act in combination with autosomal or X-linked genetic risk variants and thereby explain the increased risk of several disorders in men with KS. In support of this hypothesis of a second hit, a recently published study found that haplo-insufficiency of the X-chromosome gene, *TIMP1* in combination with specific risk variants of a paralogue gene, *TIMP3* situated on chromosome 22, might explain the increased risk of some of the cardiovascular pathology in Turner syndrome (Corbitt et al., 2018).

To date, our knowledge about genetic variants and impact on the phenotype in KS is sparse. Genetic variants of the AR gene have been investigated in relation to phenotypic traits in KS as described previously. In addition, genetic variants in autosomal genes have also been a focus of research in the recent years. Single nucleotide variants in the promoter of the *FSHB* gene (rs10835638 [c.-280G > T]) and in the FSH receptor gene *FSHR* (rs10835638 (c.-280G > T); rs6166 (c.2039G > A)) have been demonstrated to impact the level of FSH in serum in men. To evaluate whether these variants also have an impact on FSH and other endocrine parameters in men with KS, Busch et al. investigated 309 nonmosaic KS men (Busch, Tuttelmann, Zitzmann, Kliesch, & Gromoll, 2015). They found that the FSHB variant was significantly associated with serum FSH level in the untreated group of KS men; however, no effect was seen on endocrine or reproductive parameters, such as bi-testicular volume, LH, testosterone, SHBG, and estradiol. Neither did the author find any effect of the two FSHR polymorphisms on endocrine or reproductive parameters, including the FSH level. Additionally, studies of genetic variants in relation to the risk of thrombosis, which is fourfold to sixfold increased in KS (Chang et al., 2019), have also been investigated in men with KS. Erkal et al. investigated the distribution of plasminogen activator inhibitor 1 (PAI-1) gene variants in men with KS in addition to the PAI-I plasma level (Erkal, Kalayci, Palanduz, Dasdemir, & Seven, 2018). No differences were seen in the allelic frequency between men with KS and controls; however, the PAI-1 plasma levels in heterozygous polymorphism carriers were higher in men with KS compared to controls. Additional studies unraveling the impact of X-linked and autosomal genetic variants on phenotypic trait in KS are needed to shed further light on this area.

6 | THE ROLE OF COPY NUMBER VARIANTS

The impact of copy number variants (CNVs) on genetic diversity and disease susceptibility has been known for almost two decades (Iafrate et al., 2004). Whether KS is associated with an increased burden of genome-wide CNVs is unknown. CNVs in combination with the supernumerary X chromosome may lead to an increased burden of diseases, as a combination of copy number variants and other genetic factors could lead to increased burden of comorbidities, a theory proposed by Feuk, Carson, and Scherer (2006). In Turner syndrome, another sex chromosome aneuploidy, there are indications that different CNVs are more frequent, affect the phenotype and may explain some of the wide variability also within that syndrome (Prakash et al., 2016). Only a single study has been published regarding KS, investigating the prevalence of X chromosome linked CNVs in men with KS (Rocca et al., 2016). A significantly higher proportion of men with KS carried X chromosome linked CNVs compared to controls (41.5% vs. 28.6% [female]/18.6% [male]), with half of the CNVs being within regions containing genes. Furthermore, the number of X-chromosome linked CNVs per patient were higher than in controls and the CNVs were predominantly duplications (94%). Interestingly,

some of the duplicated regions encompassed genes in the pseudoautosomal region or genes escaping X-inactivation, such as *SHOX*, *CSF2RA*, *SL25A6*, *PCDH11X*, genes which have also attracted attention in studies on gene expression due to differential expression level in men with KS and their potential relation to phenotypic traits (see Epigenetics and gene expression in KS).

Microdeletions of the Y chromosome are seen in 10–15% of men with nonobstructive azoospermia or severe oligozoospermia. Azoospermia is prevalent in KS and seen in more than 95% of men with KS (Gravholt et al., 2018; Smyth & Bremner, 1998). This has fostered the question if microdeletions of the Y chromosome could be a genetic factor linked to the testicular and infertility phenotype seen in men with KS. Several studies have been performed, with a few studies finding an increased prevalence of Y chromosome microdeletions in men with KS, with the prevalence ranging from 33% to as high as 67% (Ceylan, Ceylan, & Serel, 2010; Hadjkacem-Loukil, Ghorbel, Bahloul, Ayadi, & Ammar-Keskes, 2009; Mitra et al., 2006); however, several other studies have not been able to validate these findings (Ambasudhan et al., 2003; Balkan, Tekes, & Gedik, 2008; Choe, Kim, Lee, & Seo, 2007; Lee, Kim, Kim, Kim, & Kim, 2000; Rajpert-De, Ottesen, Garn, Aksglaede, & Juul, 2011; Simoni, Tuttelmann, Gromoll, & Nieschlag, 2008; Tateno et al., 1999).

No data on the prevalence of autosomal CNVs in men with KS is available, neither are studies evaluating autosomal and X-chromosome linked CNVs impact on the phenotype. So clearly this area needs to be further evaluated.

7 | THE IMPACT OF DOSAGE SENSITIVE GENES LOCATED ON THE SEX CHROMOSOMES ON THE PHENOTYPE

Gain of an additional X chromosome is the primary genetic alteration associated with KS. Given this, the primary focus of resent research concerning genotype–phenotype relations, have been on the sex chromosomes, especially the X chromosome. The sex chromosomes evolved from an identical pair of autosomes. Through this evolutionary process the Y chromosome lost the majority of genes (retain only about 40 genes) when it gained the sex determining gene, *SRY*, whereas the X chromosome retained most of the original genes (649 genes) (Bellott et al., 2014). The X and Y chromosomes are comprised of two identical pseudoautosomal regions, PAR1 (24 genes) and PAR2 (four genes) (Helena & Morris, 2007). Due to the additional X chromosome, men with KS have an additional copy of the pseudoautosomal regions. All genes within PAR1 escape X inactivation, a process mediated by the *XIST* gene, which initiates a complex series of events eventually leading to the creation of the Barr body (Carrel & Willard, 1999; Carrel & Willard, 2005). In total, 15% of X-chromosome linked genes, including the genes within PAR1, escape inactivation, whereas the remaining 85% of the genes are transcriptionally silenced, although 10% of these genes have a variable cell-type specific expression profile (Balaton, Cotton, & Brown, 2015; Carrel & Willard, 1999; Carrel & Willard, 2005). Both genes situated in PAR1 and genes escaping X chromosomal inactivation, have been hypothesized to be prime candidates causative for the phenotype in KS; PAR1 genes due to their expression from three loci in KS (two X chromosomes and one Y chromosome), and the escape genes due to a dosage effect equal to that seen in females, but different from what is normally seen in males. In addition, several genes on the Y chromosome have been found to have identical haplotypes on the X chromosomes, indicating that these genes could also be causative for the phenotype in KS, since they would then effectively be expressed from three sites (Bellott et al., 2014).

Only one pseudoautosmal gene, *SHOX*, has so far been convincingly linked to the phenotype in KS; *SHOX* explains part of the increased height associated with KS (Ottesen et al., 2010). Another pseudoautosomal gene, *SLC25A6*, may also be linked to the phenotype in KS, although the final evidence is still missing. *SLC25A6* belongs to a mitochondrial carrier subfamily, and is involved in translocation of ADP and ATP. There are indications that *SLC25A6* is more highly expressed in KS (Belling et al., 2017; Skakkebaek et al., 2018; Zitzmann et al., 2015) and may be linked to the shorter QTc interval seen in these men (Jorgensen et al., 2015), as Zitzmann et al. found that QTc was significantly shorter in men with KS expressing higher levels of *SLC25A6* (Zitzmann et al., 2015). Interestingly, *SLC25A6* has been found to be downregulated in 45,X cells (Kelkar & Deobagkar, 2010), and females with Turner syndrome (45,X) have been demonstrated to have increased QTc interval (Trolle et al., 2013) and here presumably only copy of the *SLC25A6* gene is expressed. Other pseudoautosmal genes have been found to be differentially expressed in KS including *PPP2R3B*, *AKAP17A*, *GTPBP6*, *ZBED1* and *ASMTL* (Belling et al., 2017; Skakkebaek et al., 2018; Zitzmann et al., 2015), however an association to phenotypic traits has not been demonstrated. There is no evidence for a gene dosage effect of either escape genes or X-chromosome linked genes with identical Y haplotype, on phenotypic traits in KS, although RNA expression studies have revealed that several escape genes (*KDM5A*, *KDM5C*, *DDX3X*, *EIF1AX*, *EIF2S3*, *PRKX*, *RPS4X*, *TXLNG*, *ZFX*) are differentially expressed in KS (Belling et al., 2017; Skakkebaek et al., 2018; Zitzmann et al., 2015). However, when taking into account the Y homologs of five of these escape genes (*UTY*, *KDM5D*, *DDX3Y*, *EIF1AY*, *ZFY*), no difference in expression values were seen between KS and male controls (Skakkebaek et al., 2018).

8 | EVIDENCE FROM RNA EXPRESSION STUDIES

Evidence suggests that genes linked to the sex chromosomes may regulate gene expression throughout the entire genome as these chromosomes are enriched for genes involved in transcription and translation (Bellott et al., 2014). Recently published transcriptome studies performing genome-wide RNA-sequencing profiling of either Epstein–Barr virus transformed B lymphoblastoid cell lines, peripheral blood mononuclear cells or leucocytes from peripheral blood samples from men with KS have identified pervasive alterations in RNA

expression in a genome-wide manner, with differential gene expression of both X chromosomal as well as autosomal genes (Belling et al., 2017; Raznahan et al., 2018; Skakkebaek et al., 2018; Zhang et al., 2020; Zitzmann et al., 2015), supporting the theory raised by Bellot et al. (Bellott et al., 2014). In further support of this theory, comparative analyses of different sex chromosome aneuplodies performed in the studies by Raznahan et al. and Zhang et al. identified co-expression of sex chromosome dosage sensitive sex chromosomal genes and autosomal genes, and demonstrated, that these sex chromosome dosage sensitive sex chromosomal genes regulated specific co-expression network of sex chromosome dosage sensitive autosomal genes, with ZFX playing a key role (Raznahan et al., 2018; Zhang et al., 2020). Interestingly, Raznahan et al. found that some X chromosomal genes were upregulated with decreasing X chromosome dosage, and that Y chromosome dosage also have an impact on the expression of X chromosomal genes (Raznahan et al., 2018). These findings runs counter to previous belief regarding sex chromosome dosage compensation.

In addition, some of these transcriptome studies demonstrated altered gene expression of genes involved in biological pathways related to the immune system, energy balance and Wnt-signaling pathways, indicating that they may be deregulated in KS (Belling et al., 2017; Raznahan et al., 2018; Skakkebaek et al., 2018). These alterations are not only limited to coding RNA. Both X chromosomal and autosomal noncoding RNAs have also been demonstrated to show aberrant expression in KS, including many without known biological function (Skakkebaek et al., 2018). The implication of these alterations is unknown, however a few of the non-coding X chromosomal RNAs with aberrant expression are located close to the X inactivation center, suggesting the possibility that these may be involved in X inactivation processes (Skakkebaek et al., 2018).

The cardinal features of KS are small, firm testis histologically characterized by Leydig cell hyperplasia, degenerated, hyalinized tubule, and absence of germ cells (Aksglaede et al., 2006; Aksglaede, Skakkebaek, Almstrup, & Juul, 2011). In an attempts to understand the genetics behind the classic testicular phenotype seen in KS, a few transcriptome studies have been conducted, both on fetal, prepubertal (Winge, Dalgaard, Belling, et al., 2018) and adult (Winge, Dalgaard, Jensen, et al., 2018; D'Aurora et al., 2017; D'Aurora et al., 2015) KS testis samples, in addition to a single study on germ cells (Laurentino et al., 2019). However, some of these studies suffered from significant differences in cellularity between KS and control samples, which may question their clinical significance (D'Aurora et al., 2017; D'Aurora et al., 2015). Recent results indicate an enrichment of X chromosomal transcripts in addition to lncRNAs in fetal KS testis, but only an enrichment of lncRNAs in adult KS testis, with sparse overlap between the fetal and adult transcriptomes (Winge, Dalgaard, Belling, et al., 2018; Winge, Dalgaard, Jensen, et al., 2018). Interestingly, data from a recently published study by Laurentino et al (Laurentino et al., 2019) showed an altered DNA methylation of imprinted regions of the genome, whereas no alterations were seen in the transcriptome of germ cells from KS compared to controls. In addition to the above-mentioned studies, Viana et al. (Viana et al., 2014) investigated the transcriptome in postmortem brain tissue from a KS patient. Data from this study also revealed an altered transcriptome.

The global impact of the transcriptome associated with KS indicates that the genetics behind KS may be more complex than previously assumed. Further studies of target tissues, such as testis tissue, fat tissue, muscle tissue, brain tissue, are needed to investigate whether these alterations in RNA expression, give further support of an involvement in the phenotype.

9 | EVIDENCE FROM DNA METHYLATION STUDIES

Epigenetic mechanisms modulate gene expression and could therefore play a crucial role in the phenotype seen in KS. Studies are now emerging showing pervasive and global impact of the epigenome in KS in both peripheral blood cells and in brain tissue (Sharma et al., 2015; Skakkebaek et al., 2018; Viana et al., 2014; Wan et al., 2015; Zhang et al., 2020). Some of these studies show that KS is associated with a predominantly genome-wide hypermethylation with fewer genomic areas showing hypomethylation (Skakkebaek et al., 2018; Wan et al., 2015; Zhang et al., 2020), a pattern diametrically opposite to what have been observed in Turner syndrome (45,X) (Trolle et al., 2016; Zhang et al., 2020). The DNA methylation pattern seen in KS is unique, clearly discriminating KS from male and female controls (Skakkebaek et al., 2018). A special enrichment was observed for autosomal differentially methylated positions (DMPs) on chromosome 17, 18, 19, and 22. In addition, hypermethylated DMPs were enriched inside CpG islands and within 2 kb upstream of an island, whereas no clear picture were seen in relation to intragenic location in the study by Skakkebaek et al. (Skakkebaek et al., 2018), whereas the study by Zhang et al. did identify differentially methylated regions in the promotor region of inactivated X chromosomal genes and found these to be hypermethylated in KS. However, the methylation profile seen in KS does not seem to overlap with the expression profile (Skakkebaek et al., 2018; Zhang et al., 2020).

The biological impact of these changes in DNA methylation was assessed using functional annotation analysis revealing enrichment for terms related to diabetes, obesity, height, coronary and arterial disease, hypertension, dyslipidemia/hypercholesterolemia, bone mineral density, cancer, and others, supporting a biological effects, since these conditions are seen with increased frequency clinically in KS (Bojesen, Juul, et al., 2006; Bojesen, Kristensen, et al., 2006; Gravholt et al., 2018). Ten overlapping autosomal DMPs were found between the two studies using the Illumina Infinium 450 K array for both comparisons (i.e., KS vs. 46,XX and KS vs.46,XY) (Skakkebaek et al., 2018; Wan et al., 2015). All were hypermethylated. Seven of these DMPs corresponded to the known genes—*SPEG*, *ZNF497*, *G3BP1*, and *NSD1*. Special attention should be paid to *NSD1* as this gene is thought to be a nuclear transcription factor and histone methyltransferase enhancing transactivation of the androgen receptor, and furthermore

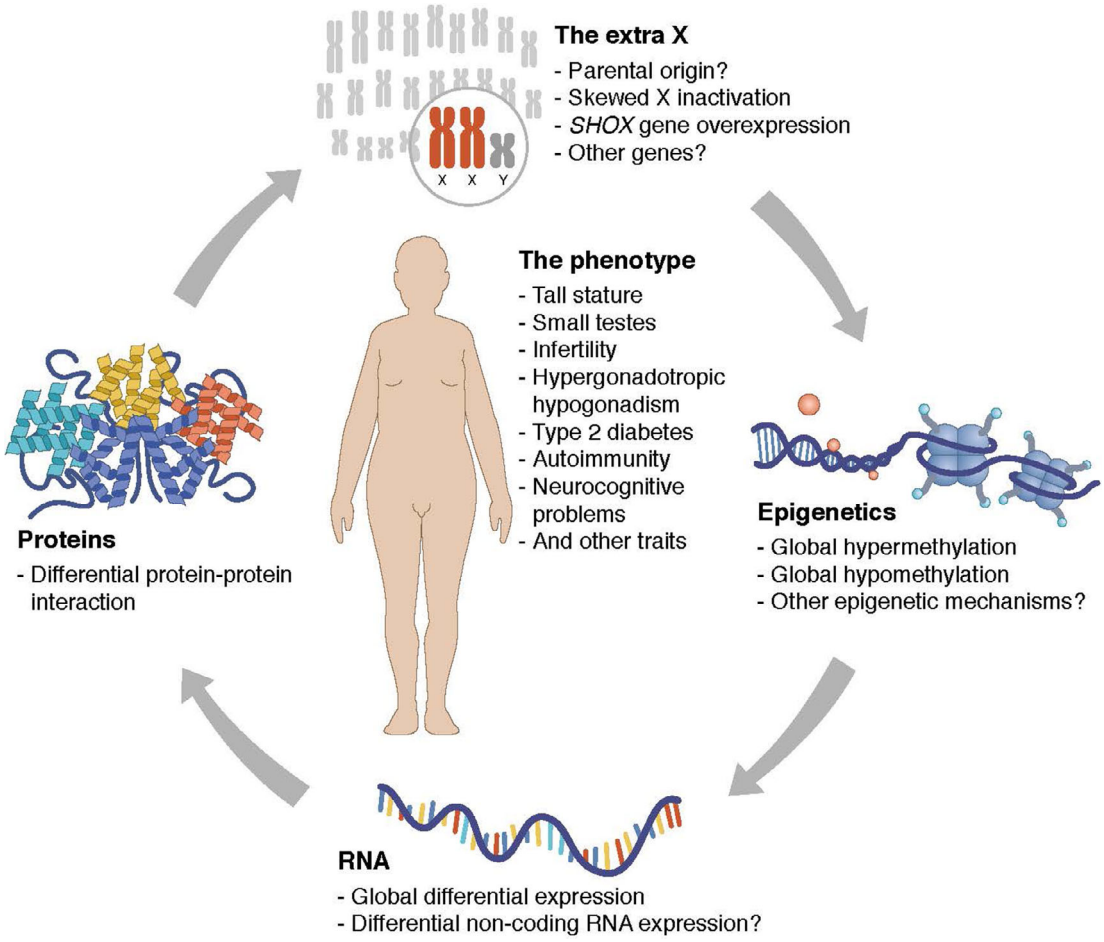

FIGURE 1 The figure depicts the current understanding of the genomics of KS incorporating recent genomic results. Arrows depict possible, but not proven pathways. Reprinted with permission from Gravholt et al. (Gravholt et al., 2018)

involved in the Soto syndrome, which bear some resemblance to some of the phenotypic traits seen in KS (Baujat & Cormier-Daire, 2007).

To conclude, evidence suggest that gain or loss of X chromosome in humans results in epigenetic instability, which may be implicated in the phenotype seen in patients with sex chromosome aneuploidies, by altering the regulation of the transcriptional and translational processes in the cells, a theory supported by a cell study (Passerini et al., 2016). In addition to DNA methylation, other epigenetic mechanism may be altered in sex chromosome aneuploidies. Jowhar et al. investigated the impact of sex chromosome aneuploidies on the global genome organization using imaging-based high-throughput chromosome territory mapping and demonstrated that the active X chromosome undergoes organizational change and decreases in size and increases in chromatin compaction with increasing number of X chromosomes (Jowhar et al., 2018). These findings are in agreement with the finding of the same chromatin conformation pattern of the inactive X chromosome in KS and in female controls (Zhang et al., 2020). The probable biological impact of these epigenetic modification found in KS calls for further studies.

10 | CONCLUSIONS

To date, the genotype–phenotype relation of KS is still largely unexplained. Only one gene –*SHOX* – has been shown to be implicated in a specific phenotypic feature of KS. Evidence from the existing literature of KS indicate that not just one single genetic mechanism can explain the phenotype and the variation in expressivity, but several candidate mechanism may be at play concurrently (Figure 1), leading to a much more complicated picture of the genomics of KS. It is evident that the additional X chromosome leads to a global genomic imbalance affecting both the epigenome and transcriptome. The overarching biological question related to KS is how to merge the understanding of the genome and epigenome with the different phenotypic manifestations. We call for future studies uniting clinical data, genomic data and basic research in attempt to understand the genetics behind KS. Undoubtedly, there will also be a need to include additional technologies, such as proteomics and metabolomics, to fully understand the complexities of KS. Unraveling the genetics of KS will be of clinical relevance as it may enable the use of polygenic risk scores to predict future disease susceptibility and

enable clinical risk stratification of KS patients in the future. In addition, a better understanding of the sex chromosome abnormalities could lead to development of new treatment strategies, such as gene therapy and targeted development of new medications.

ACKNOWLEDGMENTS

C. G. and A. S. are members of the European Reference Network on Rare Endocrine Conditions (ENDO-ERN), Project ID number 739543. The study was financially supported by research grants from The Novo Nordisk Foundation (NNF13OC0003234, NNF15OC0016474), Family Hede Nielsen foundation, Lundbeck Foundation, Augustinus Foundation, Aase og Einar Danielsen Foundation, "Fonden til lægevidenskabens fremme," University of Aarhus, Foundation of December 17, 1981 and Health Research Found of Central Denmark Region.

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How to cite this article: Skakkebæk A, Viuff M, Nielsen MM, Gravholt CH. Epigenetics and genomics in Klinefelter syndrome. *Am J Med Genet Part C*. 2020;184C:216–225. https://doi.org/10.1002/ajmg.c.31802