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Klinefelter syndrome: Expanding the phenotype and identifying new research directions

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Purpose: The purpose of this study is to summarize new data on etiology and clinical features of Klinefelter syndrome in order to derive research priorities. **Methods:** This study was conducted using critical reviews of selective topics, emphasizing less well-recognized clinical findings. **Results And Conclusions:** The phenotype of the prototypic 47,XXY case is well recognized: seminiferous tubule dysgenesis and androgen deficiency. Less well appreciated is the varied expressivity of 47,XXY Klinefelter syndrome, in particular neurological/cognitive perturbations like language and behavioral problems. Effective therapies are available. Reproductive technologies allow 47,XXY men to sire offspring through intracytoplasmic sperm injection (ICSI); however, genetic counseling is complex and success is low. Behavioral and expressive language difficulties are amenable to treatment by androgen therapy and psychological help. Early treatment may be imperative for optimal outcome. ***Genet Med* 2003:5(6):460–468.**

Key Words: *Klinefelter syndrome, seminiferous tubules, neurocognitive, language*

Klinefelter syndrome occurs in approximately 1.20 per 1000 liveborn male births, based on pooled neonatal surveys[1]; however, diagnosis is not often made at birth, making the condition appear relatively rare clinically. The phenotype of the prototypic 47,XXY case is well recognized: seminiferous tubule dysgenesis and androgen deficiency. Less well appreciated is the varied expressivity of 47,XXY Klinefelter syndromes in particular neurological/cognitive perturbations like language and behavioral problems. Only 10% of XXY boys are diagnosed prenatally, with another 25% diagnosed during childhood or adulthood.[2] The discrepancy between newborn prevalences and those identified prenatally or during childhood implies many undiagnosed cases. Almost two thirds of affected individuals might fail to be identified. If so, any effective treatment would be delayed. In particular, early treatment of learning disabilities and androgen deficiency may be imperative for optimal outcome.

To address gaps in information on Klinefelter syndrome, the National Institutes of Health sponsored a meeting, cosponsored in part by March of Dimes and Klinefelter Syndrome and Associates. Goals were stated by comoderators Joe Leigh Simpson (Baylor College of Medicine, Houston, Texas), Felix de la Cruz (NICHD, Bethesda, Maryland) and Melissa Aylstock (Klinefelter Syndrome and Associates, Roseville, California):

- Summarize current knowledge concerning the cytogenetic basis of Klinefelter syndrome, including parental origin and role X-inactivation plays in the variable phenotypes.
- Summarize the spectrum of clinical features in 47,XXY and variant forms of Klinefelter syndrome (48,XXXY; 48,XXYY; 49,XXXXY): reproductive, structural (somatic), neurological/cognitive/behavioral (learning and language disabilities).
- Update current management options for hormone deficiency, infertility, and neurological/behavioral problems.
- Enumerate the genetic counseling dilemmas that arise from detection of Klinefelter syndrome in utero (e.g., amniocentesis or chorionic villus sampling for maternal age) and from ability of Klinefelter syndrome men now to sire pregnancies through intracytoplasmic sperm injection (ICSI) and in vitro fertilization (IVF).

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- Prioritize basic and clinical research initiatives that should be undertaken. The purpose of this contribution is first to summarize data originally presented at the above mentioned conference. Thereafter, we enumerate consensus conclusions reached by participants after subsequent reflection and discussion. Research priorities thus generated by the group are offered for consideration.

CYTOGENETIC ORIGIN AND MOLECULAR PATHOGENESIS

Terry Hassold (Case Western Reserve, Cleveland, Ohio) reviewed the cytogenetic origin of trisomies in general and 47,XXY in particular. DNA polymorphisms have been used to investigate the parental and meiotic stage of origin of over 1000 autosomal and sex chromosome trisomies.[3] For autosomal trisomies, maternal errors predominate, accounting for nearly 95% of cases. Of these, over 75% are compatible with meiosis I nondisjunction, making this the single most common cause of autosomal trisomies.

In contrast, the origin of 47,XXY is more complicated. Paternal meiosis I errors (leading to $47,X^{M}X^{P}Y$) are extremely common, accounting for 50% of cases (X^{M},X of maternal origin; X^{P},X of paternal origin). The origin of maternally derived cases ($47,X^{M}X^{M}Y$) is also variable; approximately 48% are due to meiosis I errors, 29% to meiosis II errors, 7% to meiotic origins in which the specific stage is unknown, and 16% to postzygotic mitotic errors.[4]

The relationship between parental age and these different cytogenetic origins of 47,XXY is complex. Consistent with autosomal trisomies, there is no obvious increase in the age of the father among paternally derived cases. Among the maternally derived cases, an association exists with increasing maternal age; however, the effect is limited to the subset of cases originating at meiosis I. Cases originating at maternal meiosis II appear to be maternal age-independent.[4] Although we still know little of the molecular basis of autosomal trisomies, studies indicate an association between altered genetic recombination and nondisjunction.[3] Recent studies of sex chromosome trisomies indicate that this relationship holds for 47,XXY as well. Failure of the paternal X and Y chromosomes to recombine in the $X^{P}Y^{P}$ pseudoautosomal region appears to be the leading cause of cases of $47,X^{M}X^{P}Y$. Approximately two-thirds of paternally derived cases are attributable to these so-called "achiasmate" meioses.[4] Failure to recombine is also important in the genesis of $47,X^{M}X^{M}Y$ cases. Thomas et al.[5] estimated that approximately 25% of these cases involve maternal meioses in which the two X chromosomes fail to pair and/or recombine with each other. However, Thomas et al.[5] also observed another, curiously, abnormality of recombination in maternally derived cases. A small proportion of cases appeared to recombine at, or extremely close to, the centromere—normally a "dead" spot for recombination. This type of abnormality has not been observed for other human trisomies and may well be a novel mechanism of nondisjunction that is restricted to the X chromosomes.

In poly-X Klinefelter syndrome (48,XXXY; 49,XXXXY), the additional sex chromosomes almost always arise from a single parent, usually the mother.[6] 48,XXYY cases are all paternal in origin. Successive meiotic or sequential meiotic and mitotic errors are presumed to be the cytological mechanism.

Biological basis of 47,XXY was explored by Hunt Willard (Case Western Reserve, Cleveland, Ohio). The general assumption has long been that the adverse phenotype reflects those genes on the X chromosomes in excess of one that are not inactivated. Willard and Carrel are determining which of the approximately 2000 genes outside four pseudoautosomal regions of the human X chromosome escape inactivation. Of 275 genes studied, only 3 on the X long arm escaped inactivation.[7] This contrasts with almost 40 on the X short arm; thus, the X short arm is likely to be the region in which genes relevant to Klinefelter syndrome are located. The number of pivotal genes is unclear, but a useful analogy might be drawn from trisomy 21. In trisomy 21, somewhat over 200 genes have been localized, and in 1982 perhaps 50 to 100 were believed involved in the trisomic phenotype.[8] It would be logical to assume that given sequencing of chromosome 21, this number would be higher. However, no updated estimate seems to have been offered.[9] Given that the trisomy 21 phenotype is more severe than 47,XXY, it could be reasoned that the number of genes responsible for 47,XXY is fewer.

GONADAL AND HORMONAL DYSFUNCTION

Temporal development of hormonal and spermatogenic abnormalities was considered by several speakers. Neils Skakkebaek (National University Hospital of Denmark, Copenhagen, Denmark) reviewed findings indicating that in Klinefelter syndrome, testicular histology is normal or near normal in early infancy, only to show progressive loss of germ cells throughout childhood. Seminiferous tubules and Sertoli cells persist at least until puberty; however, during pubertal development Sertoli cells engulf apoptotic germ cells, resulting in the demise of tubules and subsequent hyalinization. Decades ago Skakkebaek showed that a few tubules escape this process, yielding two types of Sertoli cells: Type A, which are sex chromatin negative, and Type B, which are more often sex chromatin positive.[10,11] A marker of this apoptotic process is inhibin B, levels of which are normal or only slightly increased during childhood, but unmeasurable in adults.[12] In normal boys, inhibin-B peaks postnatally.[12] Inhibin B may thus serve as a longitudinal marker for Sertoli cell function/apoptosis in individuals with Klinefelter syndrome.

Wael Salameh (Harbor-UCLA, Torrance, California) presented histological observations in agreement with the conclusions of Skakkebaek. Near complete depletion of germ cells is observed in men with Klinefelter syndrome, despite their presence earlier in life. Given the above, the pivotal questions can be enumerated: (1) Is germ cell failure due to an XXY Sertoli cell or to an XXY germ cell? (2) What gene(s) existing on the X chromosome in increased dose (i.e., not inactivated) cause germ cell failure in XXY?

Whether germ cell and Leydig cell defects in Klinefelter syndrome are somatic or germ cell in origin was considered by Ronald Swerdloff (Harbor-UCLA, Torrance, California). This group has utilized a murine model of sex chromosome aneuploidy to study testicular development in XXY mice.[13] These animals were generated by injection of XY embryonic stem cells into XX blastocysts. Resulting female chimeras were mated with XY males and the offspring screened by Southern blotting and FISH (X and Y specific probes) to detect the XXY offspring. The resulting four XXY adult male mice showed complete absence of germ cells, with hypertrophic and hyperplastic Leydig cells; testes size was markedly decreased. However, testicular testosterone levels were not different between control (XY) and XXY mice. Sertoli cells in adult animals were not always normal; nests of abnormal cells showing scanty cytoplasm and irregularly shaped nuclei were observed. By electron microscopy, Leydig cells were increased in number and showed hypertrophy with increased smooth endoplasmic reticulum. The precise reproductive and hormonal sequelae of these findings are not known.

When temporally did testicular abnormalities arise in the XXY mice? Normal germ cells were seen in the animals at ages 1, 3, and 5 days; however, by Day 7, decreased germ cell number and testis size were observed. By Day 10, germ cell number was only 10% that of control animals. Germ cell damage was associated with positive TUNEL (terminal deoxynucleotidyl transferase (TdT)–mediated dUTP nick end labeling) staining, indicating loss of germ cells by apoptosis. Investigations to determine the chromosomal complement of Sertoli cells and germ cells in both immature and adult animals will be needed to characterize the pathogenesis of Klinefelter syndrome.

Paralleling testicular histological findings are hormonal findings in XXY humans. Pituitary gonadotropins are elevated and hyperresponsive to GnRH stimulation. Testicular responsiveness to hCG is decreased, as exemplified by decreased steroidogenesis with a block of steroid 17–20 lyase. Despite decreased steroidogenesis, Leydig cell hyperplasia occurs and the frequency of tumors is increased. These features are shared with other forms of testicular injury (irradiation, vitamin A deficiency, cryptorchidism). If gonadotropins were elevated, one would expect end organ hormones (testosterone) to be decreased. Yet Wael Salameh (Harbor-UCLA, Torrance, California) observed that at least 50% of late adolescent and early adult males with Klinefelter syndrome show serum testosterone concentrations within the normal range. Even if serum testosterone levels are normal, serum LH and FSH are almost universally elevated. It is not clear whether this situation is representative of compensated hypergonadotropic hypogonadism, in which serum testosterone levels are normalized at the expense of elevated gonadotropins, or whether it is due to partial androgen resistance.

Some Klinefelter syndrome males appear to undergo pubertal and secondary sexual development normally. Yet early hormonal treatment may still be desirable. It has been proposed that administering testosterone in the first two to three months of life or treatment of preadolescent KS boys might result in the KS phenotype becoming more normalized, thus enhancing male gender social interaction. Options for testosterone therapy include transdermal gels, patches, oral testosterone preparations, and injectables. The optimal regime is unclear, and Swerdloff emphasized the difficulty in conducting placebo-controlled studies when many patients are already convinced that treatment may be beneficial. Data are especially inadequate to determine the benefit of androgen administration on cognitive and behavioral parameters, a topic to be considered below. The appropriate androgen substitution for preadolescent boys with Klinefelter syndrome (KS) remains to be determined.

Psychosexual issues were addressed by Heino Meyer-Bahlburg (Columbia University, New York). Hypogonadism-related problems of sexual functioning in men with the karyotype 47,XXY and its variants are well documented and can be treated quite effectively by androgen replacement therapy. Less clear is the significance of case reports[14–18] describing variants in psychosexual development; in the absence of genuine population-based studies, meaningful conclusions cannot be drawn concerning whether these abnormalities are seen more commonly than in the general population.

Similarly, both observations of the 47,XXY chromosomal complement as well as published indications of decreased androgenization in later fetal development (lowered cord-blood testosterone levels and reduced penis size at birth) suggest the plausibility that disturbances could exist in basic processes underlying sexual differentiation of brain and, hence, behavior. These are likely to be modified further by difficulties in motor and language development and later language-based learning disabilities. Social interaction skills are complex and further affected by incomplete physical maturation and its psychological consequences in the untreated adolescent and adult hypogonadal male.

Existing clinical impressions concerning these problems are colored by uncertainties and biases of ascertainment. Surveys or case-control studies are needed to determine if paraphilias and gender identity disorders are truly increased among KS men. Rigorous assessment methods should be based on well-developed contemporary diagnostic systems, such as the DSM-IV-TR. Given the lack of systematic surveys of (nonsexual) psychiatric disorders in KS men, and the possible comorbidity of psychiatric and sexual disorders, a combination of the two types of surveys would be particularly useful. Also needed are comparative surveys regarding the effectiveness of various psychosocial and/or psychopharmacological treatments used in KS men with paraphilias. Such studies should specifically focus on the role of testosterone replacement or testosterone suppression in the treatment of KS with paraphilias.

Given the need for medical, mental-health, and educational services for KS men in general, and given the reported barriers to utilization of such services by the general homosexual population, a needs assessment survey of homosexual men with KS would be an important step toward improving service delivery to this subgroup.

SOMATIC ANOMALIES

Frequency of somatic anomalies in 47,XXY, 48,XXYY, 48,XXXY, and 49,XXXXY was reviewed by Dr. John Graham

(Cedars-Sinai Medical Center–UCLA, Los Angeles, California) and by Dr. Judith Hall (U. British Columbia, Vancouver, British Columbia). Both speakers concluded that there is no characteristic XXY phenotype at birth—reproductive or somatic. At birth, a small penis may furnish a clue to the diagnosis, but otherwise reproductive organs are normal. Dr. Alan Rogol (U. Virginia, Charlottesville, VA) pointed out the KS child may be born with a smaller than average penis, yet still not meet the definition of a so-called micropenis. Still, such infants might respond to testosterone, perhaps administered during the physiological "mini-puberty" observed normally around age 3 months. In the prepubertal years, XXY Klinefelter syndrome children may be impossible to distinguish physically from XY children, but often recognizable by their cognitive and behavioral problems.

Klinefelter syndrome infants give many the impression of midfacial hypoplasia and relatively small head in proportion to their tall stature with long legs. Other somatic anomalies include narrow shoulders (2-cm decrease), broad hips (1-cm increase), scoliosis and kyphosis secondary to ligament laxity (S-curve), cervical ribs, anomalous ribs, sacralization of the last lumbar vertebrae, pectus carinatum or pectus excavatum, pes planus, decreased width (25%) between the wrist and the elbow, anomalous 5th toe and 5th finger clinodactyly. Mean height is at the 75th percentile. Span is 7 cm greater than the height, and XXY men have relatively longer legs. More detailed descriptions are provided by Visootsak et al.[19]

In poly X-Klinefelter syndrome, the phenotype progressively deviates from normal as the number of X chromosomes increases.[19] In XXXY and XXXXY, the frequency of almost any given somatic anomaly is increased compared to XXY. Radioulnar synostoses are the sentinel marker, becoming more frequent and more pronounced as number of X chromosomes increases. In XXY subjects, the distance between radius and ulna is narrowed, whereas radio-ulnar fusion is increasingly observed in XXXY and XXXXY. Height decreases as the number of X chromosomes increase.[19]

The XXYY variant is not infrequently ascertained in mental hospitals and penal settings. Again, cytogenetic origin is from a single parent. XXYY individuals are often tall, having long legs and eunuchoid proportions. Peripheral vascular disease is especially frequent compared to XXY, and XXYY males also have a small penis. It is unclear if the increased criminality reflects a basic behavioral defect or is a secondary phenomenon due to impulsive behavior caused by the inability to respond verbally (see next section). Either could be confounded by tall stature, which might increase the likelihood of incarceration given fear of authorities. Several speakers have since published detailed reviews, namely Samango-Sprouse[20] and Geschwind et al.[21]

IQ AND LANGUAGE DEVELOPMENT

Neurocognitive development and language were reviewed by Bonnie Brinton (Brigham Young University, Provo, Utah), Carole Samango-Sprouse (George Washington University, Washington, DC), Dan Geschwind (UCLA School of Medicine, Los Angeles, California), and finally, Kyle Boone and Po Lu (Harbor-UCLA, Torrance, California).

In overviews, Samango-Sprouse and Brinton first concluded that insufficient IQ and language studies exist in Klinefelter syndrome; few studies are genuinely prospective. Most studies are descriptive and relatively few are even case control in design. Sample sizes are generally small. Another major pitfall is that prenatally diagnosed and treated boys are not separated from postnatally diagnosed children who are not treated, nor were family members always studied for comparison. In adolescent and older XXY males, use of hormones is not taken into account. Relatively few psychosocial studies have been conducted in the last decade.[20–25] Fewer still involve a cohort ascertained in an unbiased fashion and even these cases pose questions concerning representativeness.

The longest standing cohort is the Denver Study of Sex Chromosomal Abnormalities. This cohort began in 1964 with ascertainment through sex chromatin analysis of amniotic membranes and confirmation of full chromosomal complement through peripheral blood analysis. Many psychosocial investigations on this cohort have been reported.[24–28] In 1995, Bender et al.[25] provided information based on 11 47,XXY men, then 27.7 $\pm$ 2.76 years old. Verbal IQ (VIQ) was 90.1, performance IQ (PIQ) 94.9; mean years of education were 12.8 years. Although within the normal range, intellectual function was significantly different from sibling controls. All but one 47,XXY male was employed. However, none of the 47,XXY men were professionals, whereas 3 of 16 siblings had professional jobs; 4 of the 11 Klinefelter syndrome men were in "skilled positions," compared to 6 of 16 siblings. This Denver group emphasized that 47,XXY individuals generally fall within the normal range, albeit skewed to lower IQ compared to euploid siblings.[25]

At Harbor-UCLA Medical Center, 35 Klinefelter syndrome adolescents and adults (aged 16 to 61) self- or physician-referred for endocrinological assessment and management of hypogonadism and/or infertility were evaluated. A comprehensive neuropsychological battery was performed, and compared to 22 controls selected from the self- or physician-referred patients who presented to a university hospital clinical care. The Klinefelter patients scored significantly below controls in language skills, verbal processing speed, verbal and nonverbal executive abilities, and motor dexterity, as reviewed in detail by Geschwind et al.[21] Within the Klinefelter sample three subgroups were identified: One group ($N = 10$) had decreased verbal abilities in comparison to performance abilities (VIQ 7 or more points below PIQ). A second group ($N = 12$) had verbal abilities within 6 points of the PIQ. In a third group ($N = 12$) the perceptual ability IQ was 7 or more points less than VIQ. Deficits in language, verbal processing speed, and verbal executive skills were restricted to the lower VIQ subgroup, whereas abnormalities in motor dexterity and nonverbal executive skills were confined to the lower PIQ subgroup. Older age was significantly correlated with increased VIQ, raising the intriguing possibility that the lower PIQ subgroup primarily emerges in young adulthood. Correlative studies on

cognition and brain imaging are being performed by this group, the hypothesis being altered left-hemisphere functions in Klinefelter syndrome.[21]

Samango-Sprouse's data are derived from prenatally ascertained cases.[20] Her cohort consists of 60 XXY boys, from an upper middle class socioeconomic population; 90% of their parents had college or higher education, and these parents wittingly had their XXY child despite having had the opportunity to terminate in utero. Thus, parental concern and ability to expend resources can be assumed. In the study of Samango-Sprouse,[20] comprehensive neurodevelopmental assessments were conducted from 2 months through 7 years of age. The following conclusions have been derived. (1) Truncal hypotonia was present in 68% of the Klinefelter syndrome boys at three months of age; 15% to 20% had flattened occipital region and diminished contralateral range of motion in the neck (pseudotorticollis). Mean age at walking was 12 months. Deficits in organization and planning were noted in infants and toddlers having truncal hypotonia; avoidance of textured foods was characteristic of some children. (2) Mean IQ was 110 in this sample, with only 5% showing IQ in the superior range. (3) Speech delay was evident by 12 months. Standardized testing revealed reduction in phoneme development and difficulty with coordinating oral facial musculature, specifically lip and tongue movement. Speech delay was further defined by limited expressive vocabulary skills and limited expressive language processing. The overall result was increased risk for social interactive difficulties and peer interaction disturbances. (4) Differences existed in sensory processing, XXY infants preferring visual processing over auditory processing. Their parents described sensory dysfunction in a subset of cases. There was a tendency for gaze aversion and avoidance with overstimulation. Short-term auditory memory was poor, despite above average visual memory skill. Studying samples ascertained in different ways, other groups have arrived at similar conclusions.[22–29]

Conference attendees have discussed the proposed thesis of Samango-Sprouse and reached consensus on several tenets:

1. XXY infants show decreased truncal muscle tone and atypical gross motor skills, as shown by Samango-Sprouse.[20] Mean age of walking is typically 12 months with therapeutic intervention. Without therapy, mean age of walking is 18 months.
2. IQ is in the normal range, but shifted to the left (lower). Mean IQ for both VIQ and PIQ is usually in the low 90s to 100. KS adults are typically employed but relatively less likely to hold professional positions than their euploid siblings.
3. Klinefelter syndrome is increasingly recognized as a common cause of mental retardation, comparable to fragile X. For example, Youings et al.[30] surveyed boys aged 5 to 10 years in English special education schools and found a prevalence of full mutation FRAXA of 1 in 5,530, and the prevalence of FRAXE of 1 in 23,423; however, most had been previously diagnosed. In another study, Khalifa et al.[31] studied 670 prepubertal males initially surveyed to detect FRAX and found FRAXA in 8 boys, but in 3 of them other family members were already known. Yet 8 Klinefelter syndrome cases were detected (1.2%), and in none had the diagnosis been previously suspected.
4. Language skills are delayed, with first words spoken around 18 to 24 months versus 12 months normally. Delayed speech and language development persists during childhood, with word finding a special problem.[29] Difficulty exists in formulating sentence structure and in producing coherent narratives. Deficits exist in comprehension when language is complex or abstract; thus, written text and language processing as related to reading or composition is impeded.
5. Demeanor is passive. This makes perplexing the paradoxical behavioral outbursts observed with increasing age. Presumably this reflects frustration at being unable to cope verbally in socially vexing circumstances.

Jay Giedd (NIH, Bethesda, Maryland) discussed MRI findings that relate to these points. The brain of an XXY male is generally smaller than that of an XY male. A decrease in temporal lobe size may exist, coupled with a possible increase in the occipital region. This would be consistent with decreased auditory and increased visual receptivity, in turn leading to obtunded oral language skills. Geschwind et al.[21] are also conducting CNS visualization correlations.

Speech delay and decreased verbal IQ characteristic of XXY is more pronounced in XXXY than in XXY, again predominantly involving language expression. Findings are further increased in XXXXY, both in magnitude and frequency. IQ is decreased approximately 15 points per additional X chromosome. In 48,XXYY, IQ is usually 60 to 80.

Valuable perspectives were provided from several highly informed "consumers": Melissa Aylstock (Roseville, California), Sherryl Belinsky (South Riding, Virginia), and Dennis Kearney (Suizun, California). These three speakers presented the perspective of, respectively, parents of a young adult with XXY, parents with a preschool child with XXY, and an adult male who himself is 47,XXY. The three speakers presented their personal experience with 47,XXY and, in particular, the consequences of delayed diagnosis. One speaker voiced frustration at the school system attributing learning disabilities in XXY to "poor parenting."[11] Another emphasized continuing frustration with language difficulties.

ADULT-ONSET DISORDERS

Considerable information exist on Klinefelter syndrome, however, population based studies are lacking. Ronald Swerdloff (Harbor-UCLA, Torrance, California) has studied in detail more than 100 47,XXY subjects, ascertained in a university medical clinic. Data will soon be published detailing the prevalence of various signs and symptoms in adolescent and adult men with Klinefelter syndrome. Adrian S. Dobs (Johns Hopkins University, Baltimore, Maryland) reviewed available

knowledge concerning nonreproductive, adult onset, disorders in Klinefelter syndrome. Dr. Dobs first commented that autoimmune pathogenesis seems influenced by chronic estrogen stimulation. Not surprisingly then, the prevalence of autoimmune diseases is increased in Klinefelter syndrome compared to 46,XY men. Diabetes mellitus is increased, Type I presumably because of its association with autoimmune disease and Type II perhaps secondary to abdominal obesity; insulin resistance is also often seen in male hypogonadism, regardless of etiology. There is no evidence that testosterone treatment aggravates or causes diabetes mellitus. In fact, testosterone may constitute therapy for some autoimmune disease, diminishing the body composition abnormalities seen in Type II diabetes. Chronic estrogen stimulation may also increase antibodies related to Sjögren syndrome and systemic lupus erythematosus. Other reported disorders include thyroiditis[32] and rheumatological disorders.[33] A long recognized association between 47,XXY Klinefelter syndrome and leg ulceration and varicosities exists,[34] possibly related to an increased propensity to clotting. Case reports also exist for 47,XXY and both hypertrophic cardiomyopathy and benign neurogenic amyotrophy.[35,36] The prevalence of bipolar psychiatric disorders may be increased.[37]

A relationship seems to exist with certain neoplasias, although again robust data and population-based studies are not available. Breast cancer in particular appears markedly increased in older 47,XXY men, compared to XY men.[38] Clumps of Leydig cells commonly appear in histological examination of 47,XXY testes. Although these clumps of Leydig cells often give the appearance of an adenoma, they actually do not connote a malignant neoplastic process. Germ cell cancers have been reported, but their prevalence compared to general population is uncertain. Extragonadal germ cell cancer and mediastinal teratomas are increased; the latter is particularly striking, the frequency 34 to 40 times that of the general population.[39] One explanation invoked to explain the increased frequency of cancer in XXY is that constitutional chromosomal abnormalities and extragonadal aneuploid germ cells predispose to malignant degeneration. The analogy might be increased leukemia in Down syndrome and increased neoplasia in other sex chromosomal abnormalities.[40] More information is needed to better understand adult-onset disorders.

PREDICTED PHENOTYPE AND GENETIC COUNSELING AFTER IN UTERO DETECTION OF XXY

Joe Leigh Simpson (Baylor College of Medicine, Houston, Texas) considered genetic counseling dilemmas posed by detecting a 47,XXY fetus after prenatal genetic screening. Actually, this is relatively frequent, not unexpectedly given the maternal age effect in XXY and given advanced maternal age being the most common indication for prenatal cytogenetic diagnosis.[41] Incidence of 47,XXY increases from 0.4 per 1000 live births at maternal age 33 to 1.7 per 1000 at maternal age 40.[1] Although Down syndrome is the disorder usually sought, 47,XXY may be unwittingly detected whenever chorionic villus sampling or amniocentesis is performed for advanced maternal age.

Detection of a 47,XXY fetus during pregnancy may also occur in younger women who may be undergoing maternal serum analyte screening. However, this is less common than detection in older women undergoing chorionic villus sampling (CVS) or amniocentesis. Were second trimester analyte screening performed for the purpose of finding 47,XXY fetuses, the detection rate would be low using existing analytes and algorithms. In XXY pregnancies, maternal serum alpha fetoprotein (MSAFP) and unconjugated estriol (ìE3) show Multiple of the Medians (MoMs) almost equal that of the general population; hCG is marginally elevated (MoM 1.44). A single study exists of first trimester analytes, pooling 9,XXY, 2,XXX, and 2,XYY cases.[42] Little alteration was seen in PAPP-A (MoM 0.88) or free beta hCG (MoM 1.0) levels; serum data stratified by precise karyotype (47,XXY) alone would obviously be desirable.[42] The single most useful noninvasive technique may be nuchal translucency, which in one study was increased (MoM 2.07).[42] If an increase of this magnitude were confirmed on a larger sample, performing first trimester ultrasound (nuchal translucency, NT) would detect as many 47,XXY cases in utero as has proved to be the case in Down syndrome. In the latter, NT $>$ 95th percentile detects 75% of Down syndrome in the general population (all maternal ages) at a 5% invasive procedure (so-called false-positive) rate.[43]

Genetic counseling after detection of 47,XXY at amniocentesis or chorionic villus sampling should emphasize not only the expected phenotype but a slightly (10%) increased risk of pregnancy loss. This risk estimate is derived by comparing prevalence of 47,XXY at birth and in midtrimester amniocentesis. The various tables of Hook[1] show prevalence of amniocentesis is consistently 10% higher than in newborns. The difference is less than observed in trisomy 21 or trisomy 18, but still notable. Likewise, Hassold and Jacobs[44] estimate a higher frequency of 47,XXY in abortuses (1:300) than in liveborns.

Another important point in counseling is that predicted phenotype reflects mode of ascertainment. Cases detected coincidentally (e.g., amniocentesis for maternal age) should have a phenotype similar to those ascertained in neonatal surveys, i.e., relatively mild. By contrast, a more adverse phenotype would be expected if cases were ascertained in utero after studies undertaken for symptomatic reasons. This would be analogous to the more deleterious phenotype being observed when ascertainment occurs after an ultrasonographically evident anomaly.

Recurrence risk for a couple having had one child with 47,XXY was also considered. Few empiric data are available, but given the likely phenomenon of recurrent (autosomal) aneuploidy,[45] it seems illogical not to assume some increased risk. A useful analogy might be recurrence after trisomy 21, a disorder in which age-related meiotic nondisjunction is also associated with decreased maternal meiotic recombination. In trisomy 21, the recurrence risk is 0.75% over background; the recurrence risk is similar for trisomy 18.[46] Conversely, 47,XXY has been observed after a prior 47,XXY sibling, and a 47,XXY

child has been born to a 47,XXX mother. That recurrence of autosomal trisomy following a XXY proband has not conclusively been demonstrated to be increased probably reflects of the small data set.

GENETIC RISKS FOR OFFSPRING OF THE 47,XXY MALE

Some men with Klinefelter syndrome can now sire their own children through intracytoplasmic sperm injection (ICSI). This statement holds even if gonadotropins are elevated and even if the ejaculate is azoospermic. Sperm are usually obtained by testicular biopsy (TESA), but there is one report of ICSI using sperm from an ejaculate of an XXY man.[47]

An issue of relevance is the possibility of transmitting sex chromosomal abnormalities to offspring. Data available from the mouse models alluded to previously are relevant. In XXY animals, postmeiotic germ cell XY disomy is more frequent than in XY control mice, although far less than the theoretically expected 50%. In data of Patricia Hunt (Case Western Reserve, Cleveland, Ohio) presented at the meeting by Terry Hassold of the same institution, the frequency of XY sperm was 1.7% in XXY versus 0.2% in XY mice; the frequency of other abnormalities (non-X, non-Y) was 0.54% versus 0.05%. Hassold and Hunt concluded that the only germ cells in the XXY Klinefelter mouse capable of completing meiosis were XY, presumably having originated either by mosaicism or by trisomic rescue. Swerdloff's data revealed that in two 47,XXY mice, 80% of embryos would have been XY and only 11% XXY.[48] Both mice were $X^{M}X^{P}Y$, and Swerdloff wondered if results would have been different if cytogenetic origin had been $X^{M}X^{M}Y$.

By theoretical expectations, 50% of sperm of XXY men should be hyperhaploid (disomic), and hence, 50% of embryos should be aneuploid. As in the mouse, the observed frequency of sex chromosomal polysomy is much less. In the general population, the frequency of sex chromosomal disomy in sperm is about 0.2% in fertile XY males, 1% in infertile XY males, and 3% to 4% in XXY males.[49–52] In 47,XXY, there may also be an interchromosomal effect that is perturbing autosomes. Hennebicq et al.[53] reported higher frequencies of disomy 21 (6.2%) in 47,XXY spermatozoa compared to normal men (0.4%). No liveborn offspring of XXY fathers sired through ICSI have had sex chromosomal abnormalities. Ron-El et al.[54] collected 14 liveborns reported through August 2000, and extensive unpublished data from Brussels were presented at the meeting by Catherine Staessen (Dutch-Speaking Free University, Brussels, Belgium). Preimplantation genetic diagnosis (PGD) for Klinefelter syndrome began at that institution, and in terms of experience is the world's largest.[55] By the time of the meeting, 31 couples involving KS fathers had undergone 41 (PGD) cycles in Brussels, all involving TESA and ICSI. In 24 cycles, 39 embryos were transferred, yielding 5 fetuses with heart rates (Table 1). Among all preimplantation embryos, 59.6% were chromosomally normal. Comparing these 24 XXY cycles to 78 PGD cycles performed for X-linked recessive traits revealed in the former lower fertilization rate (54.6% vs.79.8%), increased chromosomally abnormal embryos (40.4% vs. 22.9%), and increased gonosomal abnormalities (30.0% vs. 15.2%). Updated information from the Brussels center was subsequently published.[56] In other centers, PGD in 47,XXY has also revealed chromosomal abnormalities.[57] Among abnormalities are embryos that are "chaotic," showing different chromosomal abnormalities in different cells of the same embryo.

Table 1.
Comparison of preimplantation genetic diagnosis (PGD) in couples with X-linked disease and with Klinefelter syndrome

	Indication for PGD	
	X-linked traits	Klinefelter Syndrome (Oligo or azoospermia)
No. of cycles	78	26
Mean age female (y ± SD)	32.8 ± 5.2	28.9 ± 4.1
Mean oocytes retrieved (± SD)	12.3 ± 6.3	11.8 ± 6.2
Fertilization rate in vitro (%)	79.8	54.6
Cleavage rate (%)	84.0	73.0
*Embryos (diagnosis)	546	99
XX; normal for 18	217 (39.8%)	31 (31.3%)
XY; normal for 18	204 (37.3%)	28 (28.3%)
Abnormal	125 (22.9%)	40 (40.4%)

Data from Dutch-speaking Free University of Brussels (Dr. Catherine Staessen).
Normal, Two No. 18 signals, two X or one X and one Y.

Overall, however, KS fathers utilizing ICSI have fewer than theoretically expected XY or XX disomic sperm and embryos. This could indicate either strong selection against disomic sperm or only haploid germ cells being capable of leading to a pregnancy. The latter would help explain the relatively favorable liveborn outcome. Nonetheless, the high rate of chromosomal abnormalities in preimplantation genetic embryos remains a concern. Monitoring by prenatal genetic diagnosis is recommended.

RESEARCH PRIORITIES

As a result of discussion generated at the meeting, recommendations were proposed and circulated by speakers and moderators to all invited participants. Comments were incorporated into this article. The following research priorities are thus offered:

Molecular and cytogenetic pathogenesis

1. Elucidate the behavior of X and Y chromosomes in 47,XXY during meiosis in humans and animals.
2. Determine if the decreased cytogenetic exchanges in XXY are correlated with a maternal-age effect, as observed in autosomal aneuploidies (e.g., Down syndrome).

3. Determine which of the inactivated genes on the X are responsible for adversely affecting the phenotype in XXY. Determine how they exert such actions.
4. Identify XXY subjects having only portions of the X chromosome missing, thus helping to determine critical region(s) responsible for the Klinefelter phenotype.
5. Determine the explanation for recurrent aneuploidy, if it truly exists, specifically in women who have had a fetus or child with XXY.
6. Determine if selection occurs in utero against XXY. And if so, to what extent and for what reason?

Gonadal and hormonal abnormalities

1. Determine if the absent germ cell-hyalinized tubule phenotype is the result of XXY germ cells, XXY Sertoli cells, XXY Leydig cells, or combinations thereof.
2. Determine if delayed Leydig cell failure is part of a genetic response to germ cell depletion/Sertoli cell injury or, are there specific characteristics to the failure that are intrinsic to XXY Leydig cells?
3. Examine what role androgen receptor polymorphism plays in pathogenesis of gonadal failure.
4. Determine the role androgen deficiency plays in exacerbating the XXY phenotype, both somatic and behavioral. Could androgen treatment mitigate against manifestations? Does this hold across different age groups or must any therapeutic benefit be initiated early in development?
5. Utilize animal models to further elucidate gonadal failure in human XXY. Is a factor(s) secreted by the germ cell/Sertoli cell compartment required for efficient steroidogenesis by Leydig cells?
6. Evaluate the role of the immediately postnatal hormone peak (3 months) as a predictor of testicular insufficiency in adulthood by pooling material derived from newborns from U.S. and Europe with Klinefelter syndrome.

IQ and expressive language difficulties

1. Examine the developmental history of prenatally diagnosed XXY cases.
2. Determine the relationship between decreased muscle tonus, delayed gross motor skills, and school-age learning differences.
3. Investigate the relationship between morphological brain findings as assessed by MRI and neurodevelopmental outcome, specifically frontal lobe and temporal lobe dysfunction.
4. Determine the impact of parental origin of X on neurodevelopmental outcome. Does X-skewed inactivation play a role?[59]
5. Establish what predictive indicators point to favorable or unfavorable linguistic skills, motor development, and the language-learning profile. The latter refers to predictive indicators that point to favorable or unfavorable linguistic skills, motor development, and language-learning profile.[20,21]
6. Clarify social function of XXY boys in relationship to their IQ and language capabilities.
7. Determine relationships between hormonal therapy, neurodevelopmental outcome, and time of onset of treatment. Is there a limited window of opportunity to treat?
8. Examine if animal models are helpful in addressing pathogenesis of neurocognitive abnormalities in Klinefelter syndrome.

Natural history and genetic transmission

1. Identify a cohort of prenatally diagnosed XXY cases devoid so far as possible from ascertainment bias. Collect data enabling potential confounding variables to be taken into account. Those parents who do and do not elect to terminate a pregnancy carrying a XXY fetus should be investigated in order to understand the factor(s) associated with the dilemma of a prenatal diagnosis of XXY. These data can help identify any biases that might alter generalizability of phenotype predicted on the basis of cases detected in utero by prenatal diagnosis but not terminated.
2. Establish a registry of older XXY cases that could be used to study natural history of Klinefelter syndrome in that age group, both somatic and behavioral. Collaborate with registries of patients with Klinefelter syndrome in Europe, where cytogenetic registries are already well established (e.g., Denmark).
3. Devise case finding methods (e.g., cross-sectional) that minimize ascertainment bias. This becomes particularly applicable to adult-onset disorders such as cancer, chronic leg ulcers, diabetes mellitus, and to psychiatric disorders.
4. Calculate the theoretical detection rate in utero of XXY using noninvasive first and second trimester maternal serum analyte screening and ultrasound nuchal translucency measurements. This will involve determining multiples of Medians (MoM) for maternal serum analytes in XXY.
5. Establish a registry to define empiric risk to offspring of pregnancies sired by XXY men through ICSI.

Increase communication to public and to physicians

1. Develop a public mechanism to disseminate new information to practitioners and parents.
2. Review and screen information by a "consensus-forming body" to assure quality control and accuracy of information.

References

1. Hook EB. Prevalence, risks and recurrence. In: Brock DJH, Rodeck C, Ferguson-Smith M, Eds. Prenatal diagnosis and screening. London: Churchill Livingstone, 1992:351–392.
2. Abramsky L, Chapple J. 47,XXY (Klinefelter syndrome) and 47,XYY: estimated rates of and indication for postnatal diagnosis with implications for prenatal counselling. *Prenat Diagn* 1997;17:363–368.
3. Hassold T, Hunt P. To err (meiotically) is human: the genesis of human aneuploidy. *Nat Rev Genet* 2001;2:280–291.
4. Thomas NS, Collins AR, Hassold TJ, Jacobs PA. A reinvestigation of non-disjunction resulting in 47, XXY males of paternal origin. *Eur J Hum Genet* 2000;8:805–808.
5. Thomas NS, Ennis S, Sharp AJ, Durkie M, Hassold TJ, Collins AR, et al. Maternal sex chromosome non-disjunction: evidence for X chromosome-specific risk factors. *Hum Mol Genet* 2001;10:243–250.
6. Hassold T, Pettay D, May K, Robinson A. Analysis of non-disjunction in sex chromosome tetrasomy and pentasomy. *Hum Genet* 1990;85:648–650.
7. Willard HF. The sex chromosomes and X chromosome inactivation. In: Scriver CR, Beaudet AL, Sly WS, Valle D, Eds. The metabolic and molecular bases of inherited disease, 8th edition. New York: McGraw-Hill, 2001:1191–1211.
8. Patterson D, Jones C, Scoggin C, Miller YE, Graw S. Somatic cell genetic approaches to Down's syndrome. *Ann NY Acad Sci* 1982;396:69–8.

9. Epstein CJ. Down syndrome (Trisomy 21). In: Scriver CR, Beaudet AL, Sly WA, Valle D, editors. The metabolic and molecular bases of inherited disease, 8th edition. New York: McGraw-Hill, 2001;1223–1256.
10. Skakkebaek NE. Two types of tubules containing only Sertoli cells in adults with Klinefelter's syndrome. *Nature* 1969;223:643–645.
11. Froland A, Skakkebaek NE. Dimorphism in sex chromatin pattern of Sertoli cells in adults with Klinefelter's syndrome: correlation with 2 types of "Sertoli-cell-only" tubules. *J Clin Endocrinol* 1971;33:683–687.
12. Andersson AM, Toppari J, Haavisto AM, Petersen JH, Simell T, Simell O, et al. Longitudinal reproductive hormone profiles in infants: Peak of inhibin B levels in infant boys exceeds levels in adult men. *J Clin Endocrinol Metab* 1998;83:675–681.
13. Lue Y, Rao PN, Sinha Hikim AP, Im M, Salameh WA, Yen PH, et al. XXY male mice: an experimental model for Klinefelter syndrome. *Endocrinology* 2001;14:1461–1470.
14. Davidson M. Case report: Sadistically motivated offending in an individual with chromosome constitution 47,XXY. *J Forensic Psychiat* 1994;5:177–183.
15. Herzog M, Money J. Sexology and social work in a case of Klinefelter (47,XXY) syndrome. *Mental Retard* 1993;5:191–192.
16. Money J. Amphoteric bisexual pathology evoked by solitary confinement and imprisonment in two syndromes: 47,XXY and 47,XYY. In: Money J, Ed. Biographies of gender and hermaphroditism in paired comparisons. Amsterdam, The Netherlands: Elsevier, 1991:318–343.
17. Seifert D, Windgassen K. Transsexual development of a patient with Klinefelter's syndrome. *Psychopathology* 1995;28:312–316.
18. Theilgaard A. A psychological study of the personalities of XYY- and XXY-men. *Acta Psychiatrica Scand* 1984;69:1–133.
19. Visootsak J, Aylstock M, Graham JM, Jr. Klinefelter syndrome and its variants: An update and review for the primary pediatrician. *Clin Pediatr* 2001;40:639–651.
20. Samango-Sprouse CA. The mental development in polysomy X Klinefelter syndrome (47,XXY; 48 XXXY): effects of incomplete X-activation. *Seminars Reprod Med* 2001;19:193–202.
21. Geschwind DH, Boone KB, Miller BL, Swerdloff RS. Neurobehavioral phenotype of Klinefelter syndrome. *Ment Retard Dev Disabil Res Rev* 2000;6:107–116.
22. Rovet J, Netley C, Bailey J, Keenan M, Stewart D. Intelligence and achievement in children with extra X aneuploidy: a longitudinal perspective. *Am J Med Genet* 1995; 60:356–363.
23. Rovet J, Netley C, Keenan M, Bailey J, Stewart D. The psychoeducational profile of boys with Klinefelter syndrome. *J Learn Disabil* 1996;29:180–196.
24. Bender BG, Harmon RJ, Linden MG, Robinson A. Psychosocial adaptation of 39 adolescents with sex chromosome abnormalities. *Pediatrics* 1995;96:302–308.
25. Bender BG, Linden MG, Harmon RJ. Life adaptation in 35 adults with sex chromosome abnormalities. *Genet Med* 2001;3:187–191.
26. Bender BG, Puck MH, Salbenblatt JA, Robinson A. Dyslexia in 47,XXY boys identified at birth. *Behav Genet* 1986;16:343–354.
27. Robinson A, Bender B, Linden MG. Summary of clinical findings in children and young adults with sex chromosome anomalies. In: Evans JA, Hamerton JL, Robinson A, Eds. Children and young adults with sex chromosome aneuploidy. New York: Wiley-Liss-for the March of Dimes Birth Defect Foundation, 1990:225–228.
28. Robinson A, Lubs HA, Nielsen J, Sorensen K. Summary of clinical findings: profiles of children with 47,XXY, 47,XXX and 47,XYY karyotypes. *Birth Defects Orig Artic Ser* 1979;15(1):261–266.
29. Graham JM Jr, Bashir AS, Stark RE, Silbert A, Walzer S. Oral and written language abilities of XXY boys: implications for anticipatory guidance. *Pediatrics* 1988;81: 795–806.
30. Youings SA, Murray A, Dennis N, Ennis S, Lewis C, McKechnie N, et al. FRAXA and FRAXE: the results of a five year survey. *J Med Genet* 2000;37:415–421.
31. Khalifa MM, Struthers JL. Klinefelter syndrome is a common cause for mental retardation of unknown etiology among prepubertal males. *Clin Genet* 2002;61:49–53.
32. Tojo K, Kaguchi Y, Tokudome G, Kawamura T, Abe A, Sakai O. 47 XXY/46 XY mosaic Klinefelter's syndrome presenting with multiple endocrine abnormalities. *Intern Med* 1996;35:396–402.
33. Kobayashi S, Shimamoto T, Taniguchi O, Hashimoto H, Hirose S. Klinefelter's syndrome associated with progressive systemic sclerosis: report of a case and review of the literature. *Clin Rheumatol* 1991;10:84–86.
34. Verp MS, Simpson JL, Martin AO. Hypostatic ulcers in 47,XXY Klinefelter's syndrome. *J Med Genet* 1983;20:100–101.
35. Yoshida K, Ryu T, Ogata T, Tsuji S, Tokushima T, Utsunomiya T, et al. An elderly man with Klinefelter syndrome associated with hypertrophic cardiomyopathy, sick sinus syndrome, and coronary arteriovenous fistula. *Jpn Circ J* 1998;62:222–224.
36. Matsubara S, Yoshino M, Takamori M. Benign neurogenic amyotrophy in Klinefelter's syndrome. *J Neurol Neurosurg Psychiatry* 1994;57:640–642.
37. Everman DB, Stoudemire A. Bipolar disorder associated with Klinefelter's syndrome and other chromosomal abnormalities. *Psychosomatics* 1994;35:35–40.
38. Hultborn R, Hanson C, Kopf I, Verbiene I, Warnhammar E, Weimarck A. Prevalence of Klinefelter's syndrome in male breast cancer patients. *Anticancer Res* 1997; 17:4293–4297.
39. Lachman MF, Kim K, Koo BC. Mediastinal teratoma associated with Klinefelter's syndrome. *Arch Pathol Lab Med* 1986;110:1067–1071.
40. Verp MS, Simpson JL. Abnormal sexual differentiation and neoplasia. *Cancer Genet Cytogenet* 1987;25:191–218.
41. Simpson JL. Genetic counseling and prenatal diagnosis. In: Gabbe SG, Niebyl JR, Simpson JL, Eds. Obstetrics: Normal and problem pregnancies, 4th edition. New York: Churchill Livingstone, 2001:187–219.
42. Spencer K, Aitken DA, Crossley JA. Maternal serum total hCG and free beta-hCG in the first trimester from trisomy 21 pregnancies. *Prenat Diagn* 2000;20:770–771.
43. Snijders RJ, Noble P, Sebire N, Souka A, Nicolaides KH. UK multicentre project on assessment of risk of trisomy 21 by maternal age and fetal nuchal-translucency thickness at 10–14 weeks of gestation. Fetal Medicine Foundation First Trimester Screening Group. *Lancet* 1998;352:343–346.
44. Hassold TJ, Jacobs PA. Trisomy in man. *Annu Rev Genet* 1984;18:69–97.
45. Simpson JL. Fetal Wastage. In. Gabbe SG, Niebyl JR, Simpson JL, Eds. Obstetrics: Normal and problem pregnancies. New York: Churchill Livingstone, 2002:729–753.
46. Snijders RJ, Platt LD, Greene N, Carlson D, Krakow D, Gregory K, et al. Femur length and trisomy 21: impact of gestational age on screening efficiency. *Ultrasound Obstet Gynecol* 2000;16:142–145.
47. Cruger D, Toft B, Agerholm I, Fedder J, Hald F, Bruun-Petersen G. Birth of a healthy girl after ICSI with ejaculated spermatozoa from a man with non-mosaic Klinefelter's syndrome: Case report. *Hum Reprod* 2001;16:1909–1911.
48. Bronson SK, Smithies O, Mascarello JT. High incidence of XXY and XYY males among the offspring of female chimeras from embryonic stem cells. *Proc Natl Acad Sci U S A* U S A 1995;92:3120–3123.
49. Bischoff FZ, Nguyen DD, Burt KJ, Shaffer LG. Estimates of aneuploidy using multicolor fluorescence in situ hybridization on human sperm. *Cytogenet Cell Genet* 1994;66:237–243.
50. Moosani N, Pattinson HA, Carter MD, Cox DM, Rademaker AW, Martin RH. Chromosomal analysis of sperm from men with idiopathic infertility using sperm karyotyping and fluorescence in situ hybridization. *Fertil Steril* 1995;64:811–817.
51. Guttenbach M, Martinez-Exposito MJ, Michelmann HW, Engel W, Schmid M. Incidence of diploid and disomic sperm nuclei in 45 infertile men. *Hum Reprod* 1997;12:468–473.
52. Okada H, Fujioka H, Tatsumi N, Kanzaki M, Okuda Y, Fujisawa M, et al. Klinefelter's syndrome in the male infertility clinic. *Hum Reprod* 1999;14:946–952.
53. Hennebicq S, Pelletier R, Bergues U, Rousseaux S. Risk of trisomy 21 in offspring of patients with Klinefelter's syndrome. *Lancet* 2001;357:2104–2105.
54. Ron-El R, Strassburger D, Gelman-Kohan S, Friedler S, Raziel A, Appelman Z. A 47,XXY fetus conceived after ICSI of spermatozoa from a patient with non-mosaic Klinefelter's syndrome: case report. *Hum Reprod* 2000;15:1804–1806.
55. Staessen C, Coonen E, Van Assche E, Tournaye H, Joris H, Devroey P, et al. Preimplantation diagnosis for X and Y normality in embryos from three Klinefelter patients. *Hum Reprod* 1996;11:1650–1653.
56. Staessen C, Tournaye H, Van Assche E, Michiels A, Von Landuyt L, Devroey P, et al. PGD in 47XXY KlineFelter's syndrome patients. *Hum Reprod Update* 2003;9:319–330.
57. Reubinoff BE, Abeliovich D, Werner M, Schenker JG, Safran A, Lewin A. A birth in non-mosaic Klinefelter's syndrome after testicular fine needle aspiration, intracytoplasmic sperm injection and preimplantation genetic diagnosis. *Hum Reprod* 1998; 13:1887–1892.
58. Bielanska M, Tan SL, Ao A. Fluorescence in-situ hybridization of sex chromosomes in spermatozoa and spare preimplantation embryos of a Klinefelter 46,XY/47,XXY male. *Hum Reprod* 2000;15:440–444.
59. Iitsuka Y, Bock A, Nguyen DD, Samango-Sprouse CA, Simpson JL, Bischoff FZ. Evidence of skewed X-chromosome inactivation in 47,XXY and 48,XXYY Klinefelter patients. *Am J Med Genet* 2001;98:25–31.