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KLINEFELTER SYNDROME AND OTHER SEX CHROMOSOMAL ANEUPLOIDIES IN THE REPUBLIC OF NORTH MACEDONIA

Tosho Plaseski[1], Iskra Bitoska[1], Dzuliana Mihajlovska[1], Sasha Jovanovska-Mishevska[1], Snezana Markovic-Temelkova[1], Dijana Plaseska-Karanfilska[2], Predrag Noveski[2]
[1]University Clinic of Endocrinology, Diabetes and Metabolic Disorders, Faculty of Medicine, Ss. Cyril and Methodius University in Skopje, R. North Macedonia
[2]Research Centre for Genetic Engineering and Biotechnology "Georgi D. Efremov", Macedonian Academy of Sciences and Arts, R. North Macedonia

Abstract

Chromosomal anomalies are frequent in male infertility with an incidence of 5.8%, higher than the incidence in general fertile population of 0.5%.

Klinefelter syndrome (47, XXY) is the most common disorder of sex chromosomes in humans found in 11% of patients presenting with azoospermia and in 0.7% of patients presenting with oligozoospermia.

Boys with 47,XXY are indistinguishable from other boys with normal karyotypes and are often detected when they are evaluated for infertility later in life.

Other sex chromosomal anomalies occur less frequently in infertile men.It is considered that many of the 47, XYY men are fertile and this aneuploidy occurs rarely among infertile men.

Cases of 46, XX males have also been reported. Molecular analysis shows that most of the 46, XX men have a translocation of the SRY(sex-determining gene) from the Y chromosome on the X chromosome. In our study we analysed the karyotypes of infertile men and identified 39 infertile men with sex chromosomal aneuploidies and Y chromosome micro-deletions.

Most of 39 patients had Klinefelter syndrome (31/39 or 79.5%), 4 men had 46,XYY syndrome and 4 had 46,XX syndrome.

All detected cases of chromosome aneuploidies were confirmed by cytogenetic analysis.

Keywords: Klinefelter syndrome, male infertility, chromosomal aneuploidy

Introduction

Chromosomal anomalies are frequent in male infertility with an incidence of 5.8%, higher that the incidence of chromosomal anomalies in the general fertile population of 0.5% [1].

Chromosomal anomalies can affect both, the sex and autosomal chromosomes. Gaining or losing an entire chromosome results in aneuploidy.

Klinefelter syndrome is the most common sex chromosomal anomalyin infertile men found in 11% of patients presenting with azoospermia and in 0.7% of patients presenting with oligozoospermia [2].

The etiology is related to paternal or maternal sex chromosome segregation during meiosis, resulting in 47,XXY karyotype in 90% of patients, while mosaicism 46,XY/47,XXY is present in 10% of patients. It is estimated that more than 90% of non-mosaic men are azoospermic.

Although no reliable data are available, some azoospermic men with Klinefelter syndrome may have residual spermatogenesis in some seminiferous tubules [3].

Other sex chromosomal anomialiesoccur less frequently in infertile men. 47,XYYsyndromeoccurs as a result of non-disjunctionof the paternal Y chromosome during the second meiotic division [4].

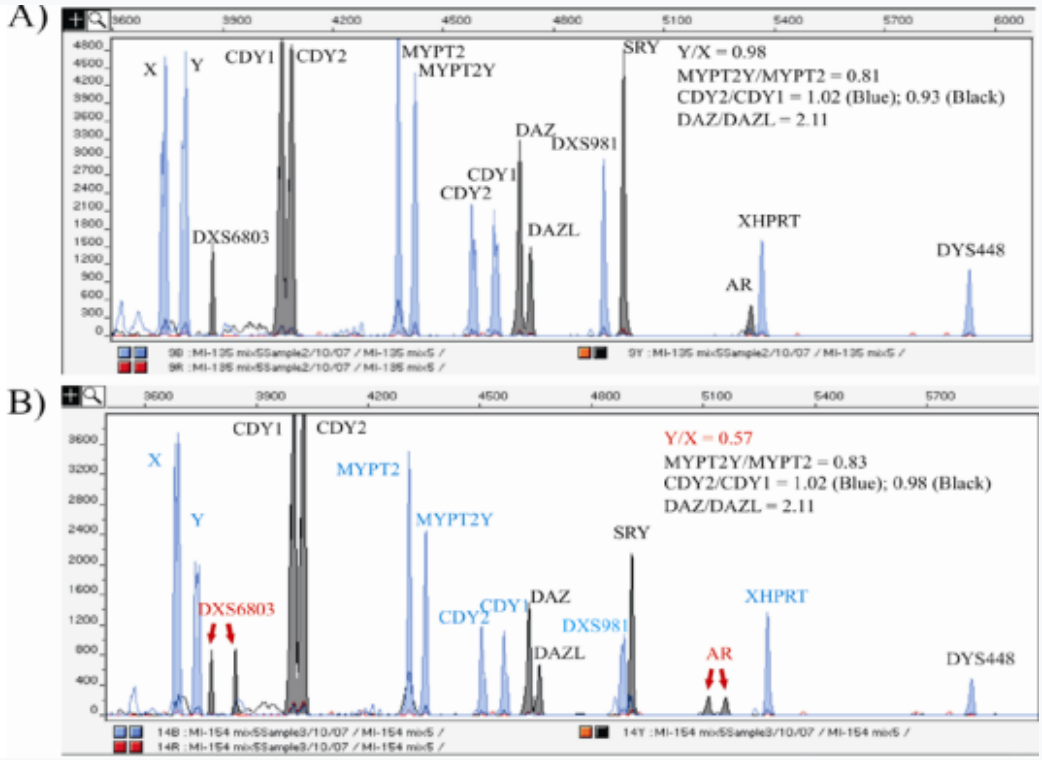
Cases of 46,XX males have also been reported, where the male phenotype is most likely due to a translocation of the *SRY*gene from the Y chromosome, or alternatively, *SRY* gene independent ways of determining the male phenotype[5,6].

Material and methods

We examined a cohort of infertile men for presence of sex chromosomal aneuploidies and Y chromosome microdeletions referred for genetic analyses to the Centre for Genetic Engineering and Biotechnology at the Macedonian Academy of Sciences and Arts.Infertility was defined as the inability to conceive after one year ofunprotected sex.

The methodology included a multiplex quantitative fluerescent-polymerase chainreaction (QF-PCR) for simultaneus detection of sex chromosomal anomalie sand deletions of the azoospermia factor (AZF) regions (AZFa, AZFb, and AZFc) ofthe Y chromosome, includingpartialAZFdeletions/duplications (Figure 1). The QF 11-plex PCR permitted the amplification of the amelogenin gene present on the X and Y chromosomes and allowed the determination of the Y/X ratio (AMEL marker).

`The AMEL marker exploits the 6-bp deletion on the X chromosomes sequence enabling amplification of specific X chromosome (106bp) and Y chromosome sequences (112 bp).All sex chromosome aneupoidies detected with QF-PCR were confirmed with a conventional karyotyping analysis [7].



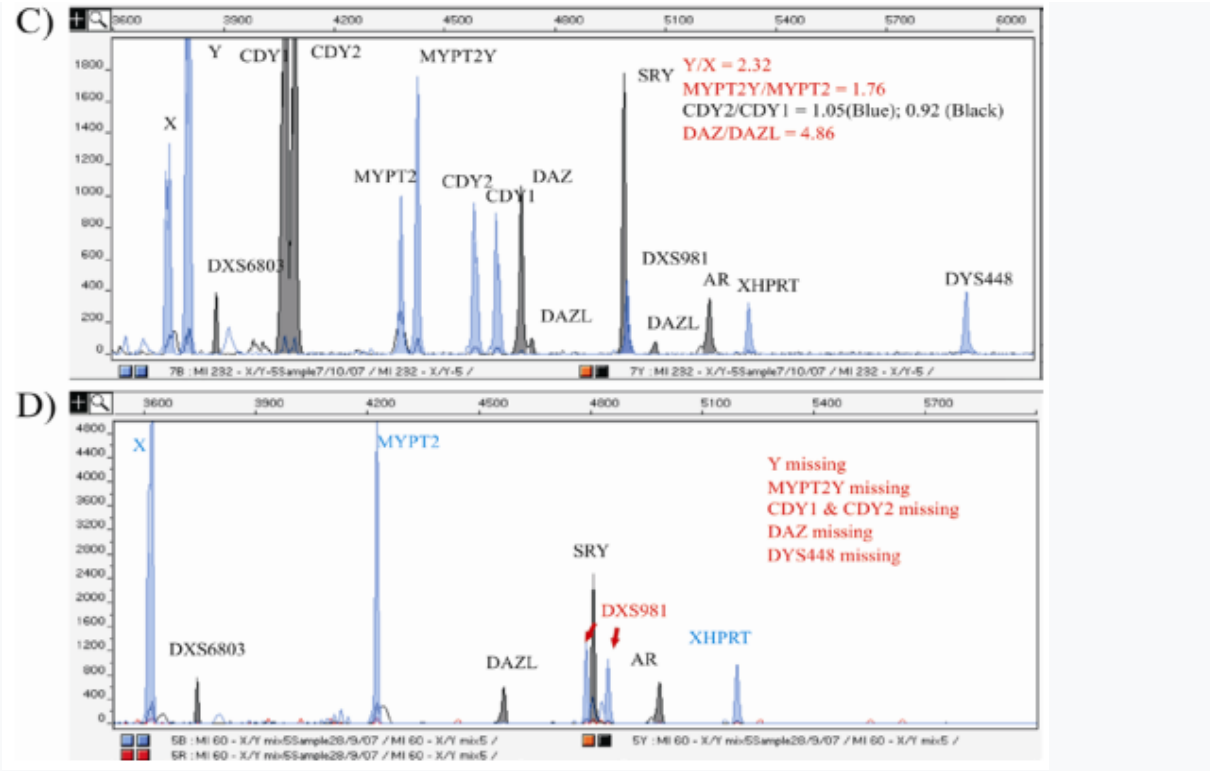


Figure 1. Representative QF PCR results.
(A) normal XY male, (B)47,XXY mal, (C) 47,XYY male and (D) 46,XX male

Results

Klinefelter Syndrome was detected by an abnormal Y/X ratio and the presence of two alleles of the X chromosome STR markers Figure 1, panel B).

Patients with 47, XYY syndrome were identified by an abnormal Y/X ratio (1.97±0.11), DAZ/DAZL (4.95±0.57) and MYPT2Y/MYPT2 (1.70±0.08), andCDY2/CDY1 ratio within normal limits (Figure 1, panel C). Patients with 46, XX syndrome were characterized by the absence of a Y fragment from the AMEL Y/X marker, DAZ and MYPT2Y fragments, as well as CDY1 and CDY2 fragments (Figure 1, panel D). The SRY fragment was present, the DYS 448 fragment was absent, and at least one of the four STR markers on the X chromosome showed two alleles.

We identified 39 men with chromosomal aneuploidy among the infertile male patients, as follows: 31 with 47, XXY syndrome(Figure 1, panel B), 4with 47, XYYsyndrome (Figure 1, panel C) and 4with 46, XX syndrome(Figure 1, panel D).

All detected cases of chromosome aneuploidies were confirmed by conventional cytogenetic analysis. All men with 47, XXYand 46, XX and one man with 47, XYY syndrome presented with azoospermia, theremaining3 patients with 47, XYY syndrome presented with oligozoospermia.

All patients with Klinefelter's syndromehad small, soft testicles up to 5 $cm^3$and high levels of gonadotropins, FSH 20.22±12.51 mIU/ml (1.6-12.0 mIU/ml) and LH 14.41±10.57 mIU/ml (0.8-6.0 mIU/ml), low testosterone levels 4.58±2.87 nmol/L (5.2-22.9 nmol/L), high-normal estradiol levels 43.10±20.43 pg/ml (5.0-56.0 pg/ml) and normal PRL levels 13.17±7.54 ng/ml (2.7-16.9 ng/ml). These results are presented in Table 1.

Table 1. Laboratory findings in patients with Klinefelter syndrome.

FSH (mIU/ml)	LH (mIU/ml)	Testosteronenmol/L	Estradiol (pg/l)	PRL (ng/ml)
20.22±12.51	14.41±10.57	4.58±2.87	43.10±20.43	13.17±16.9

Discussion

The term Klinefelter syndrome describes a group of chromosomal disorders in which there is at least one extra X chromosome in a male person. Boys with 47, XXY karyotype have variable phenotypic characteristics with no obvious facial dysmorphic features; thus, they are often indistinguishable from other boys with normal karyotypes [8]. Features that are constant in 47, XXY males are small, soft testes up to 5-6 cm^3 with elevated gonadotropins, elevated follicle stimulating hormone (FSH), luteinizing hormone (LH) and estradiol, and low to low-normal testosterone level without testosterone therapy.

A karyotype analysis of peripheral blood is the gold standard for diagnosis; and formal cytogenetic analysis is necessary to make a definite diagnosis.

47, XXY males may present with a variety of subtle clinical signs that are age-related. In infancy, males with 47,XXY may have chromosomal evaluations done for hypospadias, small phallus or cryptorchidism and/or developmental delay.

The school-aged child may present with language delay, learning disabilities, or behavioural problems. The older child or adolescent may be discovered during an endocrine evaluation for delayed or incomplete pubertal development with eunuchoid body habitus, gynecomastia, and small testes. Adults are often evaluated for infertility or breast malignancy [9].

Although most patients with Klinefelter syndrome are infertile, there have been a few reports of pregnancy without assisted medical technology, typically in mosaic cases. With the introduction of intracytoplasmic sperm injection, which involves sperm extraction from the testicular tissue of patients with nonmosaic Klinefelter syndrome, some 47, XXY men will have a chance of fathering a child. Thus, testicular sperm extraction and intracytoplasmic sperm injection may be considered in males with azoospermia and Klinefelter syndrome (10).

Men with 47,XXYsyndrome have an increased risk of acquiring breast cancer, with relative risk exceeding 200 times. This may be due the estradiol-to-testosterone ratio being several fold higher than in 46,XY men or possibly due to an increased peripheral conversion of testosterone to estradiol in men with Klinefelter syndrome [11]. Associated endocrine complications include diabetes mellitus, hypothyroidism and hypoparathyreoidism [1].

Autoimmune diseases, such as systemic lupus erythematosus, Sjogren syndrome and rheumatoid arthritis are more common in men with Klinefelter syndrome, with frequencies similar to those in females.

Development of varicose veins and leg ulcers may result from venous stasis [13]. Decreased bone density occurs in 25% of patients with Klinefelter syndrome, possibly reflecting the impact of decreased bone formation, increased bone resorption and/or hypogonadism [14].

Androgen replacement therapyshould begin in puberty at around age 12, with a dose increase aiming to achieve age appropriate serum concentrations of testosterone, estradiol, FSH and LH. Androgen replacement promotes normalization of body proportions or development of normal secondary sex characteristics, but does not treat infertility, gynecomastia, and small testes.

Testoserone replacement also results in general improvement in behaviorand work performance [15].

Testosterone also has beneficial long-term effects of reducing the risk of osteoporosis, autoimmune disease, and breast cancer [16].

Other sex chromosomal defects occur less frequently in infertile men. It is considered that 47, XYY men are fertileand this aneuploidy rarely occurs among infertile men. Similar to Klinefelter syndrome, gonadal mosaicism is thought to occur in 47, XYY men.

As a result of the competition process of germ cell lines, in which 46,XY cells have a selective advantage over aneuploid cells, normal spermatocyte development occurs resulting in most 47,XYY men being fertile [4].

46, XX men occur less frequently. These men are azoospermic, and 10% present with ambiguous genitalia or hypospadias. Molecular analyses show that most of the XX men have a translocation of a small portion of the Y chromosome that contains *SRY* (sex-determining gene) on the X chromosome[17].

Conclusion

Patients with Klinefelter syndrome and other sex chromosomal aneuploidies are often diagnosed in adulthood when they experience reproduction difficulties and present for infertility evaluation.

When evaluating male infertility, Klinefelter syndrome and other sex chromosomal abnormalities should be considered.

The most common sex chromosomal anomaly in infertile Macedonian men was Klinefelter syndrome 47,XXY found in about 80% of cases with sex chromosome anomaly.

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