sciendo

10.2478/AMB-2021-0051

ASSOCIATION OF X CHROMOSOME ABERRATIONS WITH MALE INFERTILITY

S. Xharra[1], E, Behluli[2], A. Moder[3], H. Nefic[4], R. Hadziselimovic[4], G. Temaj[5]

[1]Regional Hospital – Prizren, Kosovo
[2]Medical Faculty, University of Prishtina, Kosovo
[3]Center for Clinical Research Altenbergerstrasse, Johannes Kepler University of Linz, Austria
[4]Faculty of Science, University of Sarajevo, Bosnia and Herzegovina
[5]Human Genetics, College UBT, Faculty of Pharmacy, Prishtina, Kosovo

Abstract. *Male infertility is caused by spermatogenetic failure, clinically noted as oligo- or azoospermia. Approximately 20% of infertile patients carry a genetic defect. The most frequent genetic defect leading to azoospermia (or severe oligozoospermia) is Klinefelter syndrome (47, XXY), which is numerical chromosomal abnormality and Y- structural chromosome aberration. The human X chromosome is the most stable of all human chromosomes. The X chromosome is loaded with regions of acquired, rapidly evolving genes. The X chromosome may actually play an essential role in male infertility and sperm production. Here we will describe X chromosome aberrations, which are associated with male infertility.*

Key words: *Klinefelter syndrome, azoospermia, chromosome aberration, androgen receptors, Y chromosome, X chromosome*

Corresponding author: *Dr. Gazmend Temaj, Human Genetics, College UBT, Faculty of Pharmacy, Kosovo Kalabria, 10000 Prishtina, Kosovo, tel.: 00383-45-803450, e-mail: gazmend.temaj@ubt-uni.net, gazmendtemaj2003@yahoo.com*

RECEIVED *11 August 2020,* ***ACCEPTED*** *22 September 2020*

INTRODUCTION

Male infertility is caused by spermatogenetic failure, clinically noted as low sperm count known as oligospermia or absence of sperm in the ejaculate known as azoospermia [1, 2].

The most frequent genetic causes of severe oligo- or azoospermia are Klinefenter syndrome (47, XXY) and microdeletions of the AZF (Azoospermia factor) regions on the long arm of the Y chromosome [3].

It is well reported that more and more genes are associated with spermatogenic failure in males. For example, autosomal genes NR5A1 [4, 5] and DMRT1 [6] in heterozygote mutations are causative; sex-chromosomal genes which are suspected to cause male infertility are TEX11 [7] and RHOX [8].

The human X and Y originate from ancestral homologous chromosome pair, which during evolution lost homology due to progressive degradation of chromosome Y [9, 10].

Females inherit one X chromosome from each parent; in contrast, males inherit X chromosome from the mother and Y chromosome from the father. The dosage of X chromosome in the female is compensated through inactivation of one of the two X chromosomes [11, 12, 13].

The supernumerary of X chromosomes 47,XXY and 47,XXX are inactivated in the same manner. Disteche, in 1995, reported that 10% of human genes "escape" from X-inactivation [14].

The short regions of X and Y chromosome PAR1 and PAR2 (pseudo autosomal regions) behave like an autosome and recombine during meiosis [15, 16, 17].

X chromosome aneuploidy

The first human chromosomes were visible by Tijo and Levant [18], three years later a chromosomal aberration known as Klinefelter syndrome was identified [19].

The most common sex chromosome aneuploidies associated with infertility are: 47,XXY (Klinefelter syndrome) (1 in 500 newborn boys) and 45,X (Turner syndrome) (1 in 2500-3000 newborn girls). Two other sex chromosome aneuploidies 47,XYY (1 in 1000 newborn boys) and 47,XXX (Triplet X syndrome) (1 in 1000 newborn girls) have little effect on fertility [20, 21, 22].

MEIOSIS AND XY PAIRING

Meiotic cell division is necessary to produce germ cells for female and male. Haploid DNA content in germ cells is achieved by one round of DNA replication, followed by two successive rounds of cell division (meiosis).

Autosomal chromosomes are homologous along their entire length; but X and Y chromosomes are not homologous save for the short segments at the end of each chromosome arm, the PARs [17].

X and Y chromosomes differ in size and gene content, homology for X –Y chromosomes, exist only in PARs (pseudoautosomal regions) [23, 24].

X-autosome translocations in males

Meiosis in male with X-autosome translocations is typically affected due to failure in XY pairing; it is shown that practically all males with X-autosome translocations are infertile due to spermatogenic arrest [25, 26].

In cases of X-autosome translocations, the formation of quadrivalents was proposed, in which the PARs of the Y chromosome associate with the homologous parts of the X chromosome on the derivative X chromosome and the derivative autosome [25, 26].

In this context, also Y-autosomal translocations, with an autosomal breakpoint other than an acrocentric short arm, may result in the disruption of the sex vesicle leading to infertility [27].

X-chromosomal deletions

According the array-CGH and SNP (single nucleotide polymorphysem) it is possible to identify and analyze submicroscopic deletion and duplication, these are analyzed on a large scale in many diseases and are termed copy number variations (CNVs). The CNVs add to the normal variation in our genome and that the majority of CNVs has probably no or little relevance for disease [28].

Androgen receptor (AR)

Mutations in the X-linked AR gene cause three different diseases (1) androgen insensitivity syndrome (AIS) [29] (2) spinal and bulbar muscular atrophy (SBMA or Kennedy disease) [30] and (3) prostate cancer [31, 32].

AR mutations may lead to complete androgen insensitivity (CAIS) with a female phenotype in karyotypic males, partial forms (PAIS) in patients with ambiguous genitalia or mild forms (MAIS) in men with hypospadias, gynecomastia and spermatogenic impairment [33, 34].

Two polymorphisms, the CAG and GGN polymorphisms, located in exon 1 of the AR gene, code for polyglutamine and polyglycine stretches respectively. Longer lengths of the CAG repeat are associated with decreased transcriptional activity of the AR protein in vitro, suggesting that longer polyglutamine tracts may be related to male infertility [35, 36].

Thus, the CAG repeat length has been extensively studied for their role in male infertility. However, overall statistically significant, but very small and thus clinically irrelevant differences have been determined between infertile men and controls [36, 37].

Variations of the GGN polymorphism are frequently found in the general male population and are not associated with male infertility [38, 39].

TEX11

The *TEX11* (testis-expressed 11) gene encodes a protein critical for male germ cell meiotic DNA recombination [40].

Tex11-knockout male mice exhibit azoospermia with meiotic arrest at the pachytene stage due to an inability to repair double-strand DNA breaks [40]. Recently, Yatsenko et al. identified *TEX11* as a new genetic marker for spermatogenic arrest in men with idiopathic infertility [7].

RHOX

The X-linked *RHOX* cluster encodes as set off homeobox genes that are selectively expressed in the reproductive tract. Members of the *RHOX* cluster regulate target genes important for spermatogenesis promote male fertility in mice. Studies show that demethylating agents strongly upregulate the expres-

sion of mouse Rhox genes, suggesting that they are regulated by DNA methylation.

In human RHOX genes, whether DNA methylation directly regulates RHOX gene transcription and how this relates to human male infertility is unknown.

Results from Richardson et al 2014 [41] indicated that DNA methylation is necessary and sufficient to silence human *RHOX* gene expression. To determine whether *RHOX* cluster methylation associates with male infertility, Richardson et al 2014 [41] evaluated the methylation status of *RHOX* genes in sperm from a large cohort of infertility patients. Linear regression analysis revealed a strong association between RHOX gene cluster hypermethylation and three independent types of semen abnormalities. Hypermethylation was restricted specifically to the *RHOX* cluster; Richardson et al 2014 [41] did not observe genes immediately adjacent to it on the X chromosome. Our results strongly suggest that human *RHOX* homeobox genes are under an epigenetic control mechanism that is aberrantly regulated in infertility patients. Richardson et al 2014 [41] propose that hypermethylation of the *RHOX* gene cluster serves as a marker for idiopathic infertility and that it is a candidate to exert a causal role in male infertility [41].

Busada et al 2016 [42] described a novel germ cell-specific X-linked reproductive homeobox gene (*Rhox13*) that is upregulated at the level of translation in response to retinoic acid (RA) in differentiating spermatogonia and preleptotene spermatocytes. They hypothesize that *RHOX13* plays an essential role in male germ cell differentiation, and have tested this by creating a *Rhox13* gene knockout (KO) mouse.

Busada et al 2016 [42] indicate that *RHOX13* is not essential for mouse fertility in a controlled laboratory setting, but that it is required for optimal development of differentiating germ cells and progression of the first wave of spermatogenesis.

Transcription factor *RHOX10* is critical for initiation of spermatogonial stem cell (SSCs). Loss of the 33 gene X-linked homeobox gene cluster that includes Rhox10 causes progressive spermatogenic decline. Song et al 2016 [43] show that Rhox10 drives SSC generation by promoting pro-spermatogonia differentiation. Rhox10 regulate gene migration and promote the migration of pro-spermatogonia into SSC niche [43].

The potential role of gene RHOX in human infertility was addressed by sequencing all RHOX exons in a group of 250 patients with severe oligozoospermia. Borgmann et al., 2016 [8] show two mutations in RHOXF1 (c.515G>A and c.522C>T) and four in RHOXF2/2B (-73C>G, c.202G>A, c.411C>T and c.679G>A), of which only one (c.202G>A) was found in a control group of men with normal sperm concentration. Functional analysis demonstrated that c.202G>A and c.679G>A significantly impaired the ability of RHOXF2/2B to regulate downstream genes. By combining clinical data with in vitro functional analysis, Borgmann et al., 2016 [8] suggest that the RHOX transcription factors and some of their downstream genes represent new potential targets to be used for the diagnostics and treatment of male infertility.

CONCLUSION

X chromosome plays a predominant role in spermatogenesis. Several X-chromosomal aberrations are known to cause male infertility; this is documented by many authors. The genetic aberration ranges from an additional X chromosome found such as in Klinefelter syndrome to point mutations in genes recently published. Furthermore, patients with spermatogenic aberration carry a higher CNV on the sex chromosomes compared with fertile males. The genes TEX11 or RHOXF1 and RHOXF2/2B emphasize the importance of the X chromosome for male gonadal development. Furthermore the X chromosome aberration such as point mutation which occurred during spermatogenesis in chromosome X is known as X-linked developmental genes, in male chromosome, containing a multitude of genes orchestrating different stage of male gonadal and especially sex cell development.

REFERENCES

1. Meschede D, Lemcke B, Behre HM et al. Clustering of male infertility in the families of couples treated with intracytoplasmic sperm injection. Hum Reprod 2000, 15(7):1604-1608.
2. Zorrilla M & Yatsenko AN. The genetics of infertility: current status of the field. Curr Genet Med Rep. 2013, 1, 247-260.
3. Behre HM, Bergmann M, Simoni M et al. Source Endotext [Internet]. South Dartmouth (MA): MDText.com, p. 2000, 2015.
4. Bashamboo A, Ferraz-de-Souza B, Lourenço D et al. Human male infertility associated with mutations in NR5A1 encoding steroidogenic factor 1. Am. J. Hum. Genet. 2010, 87, 505-512.
5. Ferlin A, Rocca MS, Vinanzi C et al. Mutational screening of NR5A1 gene encoding steroidogenic factor 1 in cryptorchidism and male factor infertility and functional analysis of seven undescribed mutations. Fertil Steril. 2015, 104, 163. e1-169.e1.
6. Lopes AM, Aston KI, Thompson E et al. Human spermatogenic failure purges deleterious mutation load from the autosomes and both sex chromosomes, including the gene DMRT1. PLoS Genet. 2013, 9, e1003349.
7. Yatsenko AN, Georgiadis AP, Röpke A et al. X-linked TEX11 mutations, meiotic arrest, and azoospermia in infertile men. N Engl. J Med. 2015, 372, 2097-2107.
8. Borgmann J, Tüttelmann F, Dworniczak B et al. The human RHOX gene cluster: target genes and functional analysis of gene variants in infertile men. Hum. Mol. Genet. 2016, 25, 4898-4910.

9. Hughes JF, Skaletsky H, Koutseva N et al. Sex chromosome-to-autosome transposition events counter Y-chromosome gene loss in mammals. Genome Biol. 2015, 16, 104.
10. Charlesworth D, Charlesworth B. Sex chromosomes: evolution of the weird and wonderful. Curr Biol. 2005, 15, R129-R131. (doi:10.1016/j.cub.2005.02.011)
11. Lyon M. Gene action in the X-chromosome of the mouse (Mus musculus L.). Nature. 1961, 190, 372-373.
12. Kalantry S, Purushothaman S, Bowen RB et al. Evidence of Xist RNA-independent initiation of mouse imprinted X-chromosome inactivation. Nature. 2009, 460, 647-651.
13. Barr M, Bertram E. A morphological distinction between neurones of the male and female, and the behaviour of the nucleolar satellite during accelerated nucleoprotein synthesis. Nature. 1949, 163, 676.
14. Disteche CM. Escape from X inactivation in human and mouse. Trends Genet. 1995, 11, 17-22.
15. Burgoyne PS. Genetic homology and crossing over in the X and Y chromosomes of Mammals. Hum. Genet. 1982, 61, 85-90.
16. Polani PE. Pairing of X and Y chromosomes, non-inactivation of X-linked genes, and the maleness factor. Hum. Genet. 1982, 60, 207-211.
17. Helena Mangs A, Morris BJ. The human pseudoautosomal region (PAR): origin, function and future. Curr Genomics. 2007, 8, 129-136.
18. Tjio JH, Levan A. The chromosome number of man. Hereditas. 1956, 42, 1-6.
19. Jacobs PA, Strong JA. A case of human intersexuality having possible XXY sex-determining mechanism. Nature. 1959, 183, 302-303.
20. Rives N, Simeon N, Milazzo JP et al. Meiotic segregation of sex chromosomes in mosaic and non-mosaic XYY males: case reports and review of the literature. Int J Androl. 2003, 26, 242-249.
21. Otter M, Schrander-Stumpel CTRM, Curfs LMG. Triple X syndrome: a review of the literature. Eur J Hum Genet. 2010, 18, 265-271.
22. Kim IW, Khadilkar AC, Ko EY, Sabanegh ES. 47,XYY syndrome and male infertility. Rev Urol. 2013, 15(4):188-196.
23. Charlesworth B, Charlesworth D. The degeneration of Y chromosomes. Phil. Trans. R. Soc. Lond. B. 2000, 355, 1563-1572.
24. Heard E, Turner J. Function of the sex chromosomes in mammalian fertility. Cold Spring Harb Perspect Biol. 2011, 3(10):a002675.
25. Assche E Van, Bonduelle M, Tournaye H et al I. Cytogenetics of infertile men. Hum Reprod. 1996, 11 (Supplement 4):1-24.
26. Ma S, Yuen BH, Penaherrera M et al. ICSI and the transmission of X-autosomal translocation: a threegeneration evaluation of X;20 translocation: case report. Hum Reprod. 2003, 18(7):1377-1382.
27. Röpke A, Stratis Y, Dossow-Scheele D et al. Mosaicism for an unbalanced Y;21 translocation in an infertile man: a case report. J Assist Reprod Genet. 2013, 30(12):1553-1558.
28. Redon R, Ishikawa S, Fitch KR et al. Global variation in copy number in the human genome. Nature. 2006, 444, 444-454.
29. McPhaul MJ, Marcelli M, Zoppi S et al. Mutations in the ligand-binding domain of the androgen receptor gene cluster in two regions of the gene. J Clin Invest. 1992, 90, 2097-2101.
30. Spada AR La, Wilson EM, Lubahn DB et al. Androgen receptor gene mutations in X-linked spinal and bulbar muscular atrophy. Nature. 1991; 352: 77-79.
31. Röpke A, Allhoff E, Wieacker P. Mutationen des androgenrezeptor-gens als mögliche ursache der antiandrogenresistenz beim prostatakarzinom. Journal of Reproductive Medicine and Endocrinology 2004, 1(3):194-201.
32. McCrea E, Sissung TM, Price DK et al. Androgen receptor variation affects prostate cancer progression and drug resistance. Pharmacol Res. 2016, 114, 152-162.
33. Wilson JD, Harrod MJ, Goldstein JL et al. Familial incomplete male pseudohermaphroditism, type 1. Evidence for androgen resistance and variable clinical manifestations in a family with the Reifenstein syndrome. N Engl. J Med. 1974, 290, 1097-1103.
34. Brinkmann AO. Molecular basis of androgen insensitivity. Mol Cell Endocrinol. 2001, 179(1-2):105-109.
35. Gao T, Marcelli M, McPhaul MJ. Transcriptional activation and transient expression of the human androgen receptor. J Steroid Bioch Mol Biol. 1996, 59(1):9-20.
36. Pan B, Li R, Chen Y et al. Genetic association between androgen receptor gene CAG repeat length polymorphism and male infertility: a meta-analysis. Medicine. 2016, 95, e2878. (doi:10.1097/ MD.0000000000002878)
37. Meyts ERD, Leffers H, Petersen JH et al. CAG repeat length in androgen-receptor gene and reproductive variables in fertile and infertile men. Lancet. 2002, 359, 44-46.
38. Ferlin A, Bartoloni L, Rizzo G et al. Androgen receptor gene CAG and GGC repeat lengths in idiopathic male infertility. Mol Hum Reprod. 2004, 10, 417-421.
39. Ruhayel Y, Lundin K, Giwercman Y et al. Androgen receptor gene GGN and CAG polymorphisms among severely oligozoospermic and azoospermic Swedish men. Hum Reprod. 2004, 19, 2076-2083.
40. Adelman CA, Petrini JHJ. ZIP4H (TEX11) deficiency in the mouse impairs meiotic double strand break repair and the regulation of crossing over. PLoS Genet. 2008, 4, e1000042. (doi:10.1371/journal.pgen.1000042)
41. Richardson EM, Bleiziffer A, Tuttelmann F et al. Epigenetic regulation of the RHOX homeobox gene cluster and its association with human male infertility. Hum Mol Genet. 2014, 23(1):12-23
42. Busada JT, Velte EK, Serra N et al. Rhox13 is required for a quantitatively normal first wave of spermatogenesis in mice. Reproduction. 2016, 152(5):379-388.
43. Song WH, Bettegowda A, Lake BB et al. The Homeobox Transcription Factor RHOX10 Drives Mouse Spermatogonial Stem Cell Establishment. Cell Rep. 2016, 17(1):149-164