Review Article

Azoospermia Factor C Subregion of the Y Chromosome

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Abstract

The azoospermia factor (AZF) region on the Y chromosome consists of genes required for spermatogenesis. Among the three subregions, the AZFc subregion located at the distal portion of AZF is the driver for genetic variation in Y chromosome. The candidate gene of AZFc is known as deleted in azoospermia gene, which is studied with interest because it is involved in germ cell development and most frequently deleted genes leading to oligozoospermia and azoospermia. Recently, two partial deletions in AZFc gr/gr and b2/b3 are characterized at the molecular level which showed homologous recombination between amplicons, affecting spermatogenesis process. There are novel methods and commercially available kits for accurate screening and characterization of microdeletions. It is important to detect the AZFc microdeletions through genetic screening and counseling those infertile men who planned to avail assisted reproduction techniques such as undergoing intracytoplasmic sperm injection or *in vitro* fertilization.

Keywords: *Azoospermia factor c microdeletion, azoospermia factor region, b2/b3 deletion, deleted in azoospermia, gr/gr deletion, male infertility, sequence-tagged site polymerase chain reaction, Y chromosome*

Introduction

Infertility is defined as the inability to conceive or produce an offspring after 1 year (time to pregnancy) of regular unprotected intercourse.[1,2] Approximately 15% of couples are affected with infertility,[3-5] in which male factor infertility accounts for approximately 50% with genetic abnormalities alone account for 15%–30% of male factor infertility.[6] Genetic factors contribute to male infertility by influencing a variety of physiological processes including hormonal homeostasis, spermatogenesis, and sperm quality. Male infertility screening is carried out using semen analysis according to standard reference values of the World Health Organization.[7]

Genetic cause, especially chromosomal aberrations and microdeletions of the Y chromosome, is considered to be major and well characterized cause of male infertility for azoospermia and severe oligozoospermia males.[6,8] Mostly the male infertility is a *de novo* event of genetic origin, which originates during the spermatogenesis.[9] More than 4000 genes are said to be involved in human spermatogenesis.[10] Recognition of azoospermia factor (AZF) region on the long arm of the Y chromosome is the second most common accountable genetic cause of spermatogenic failure. Molecular screening of Y chromosome microdeletions explained the tripartite (AZFa, AZFb, and AZFc) organization which regulates spermatogenesis.[11] It has been reported that the AZF region harbors 12 genes/gene families by DNA sequencing analysis.[12,13]

Worldwide, several studies in recent years have investigated the association of infertile male phenotype with selected gene sequence polymorphism. The heterogeneity of the phenotype due to selection criteria, population structure, ethnic background, environmental influence, and epigenetic factors are the most important limitations. Therefore, in this review article, we present a brief overview of the AZFc subregion as deletion in this

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region is a genetic risk factor for spermatogenic failure and to understand the genetic complexity of the AZFc sub-region of human Y chromosome.

Azoospermia Factor C Region

The AZFc subregion has been studied extensively as the frequency of AZFc deletion is high among azoospermic and oligozoospermic men[11] which results in severe spermatogenic failure were sperm count is <5 million/ml of semen in nonobstructive males. In recent years, partial deletions in the AZFc subregion have been studied at the molecular level.[14] Four partial deletions have been identified which are b2/b4, gr/gr, b2/b3, and b1/b3.[15-17] Among these four, gr/gr deletion is the most common deletion occurring because of recombination.[18]

AZFc subregion has very long repeat units, called amplicons which contains eight multicopy gene families, namely deleted in azoospermia (DAZ), basic protein Y2, chromodomain on Y (CDY1), golgi autoantigen, golgin subfamily a2 like Y, chondroitin sulfate proteoglycan 4 like Y, testis-specific transcript, Y (TTTY)-linked 3, TTTY4, and TTTY17.[13,16,19] Nonallelic homologous recombination (NAHR) occurs between amplicons which include deletions, duplication, or both leads to copy number alteration of genes in the AZFc subregion.[20] DAZ family genes were the first genes to be identified in azoospermic cases.[19] DAZ protein polymorphic expression denoting the diverse activities of each DAZ copy.[21] It is necessary to study the DAZ copies and other related gene deletion pattern to know the association between AZFc polymorphism and infertility in males.[22] Furthermore, there is an effect of copy number variations (CNVs) of all the eight gene families on male infertility which is rarely reported.[23,20] The reports suggest that due to the reduction and/or increase in gene number or simultaneous reduction and/or increase in gene copy number may be the reason for spermatogenic failure due to CNVs.[20]

Genetic Organization and Recombination Mechanism

Several authors have reported that deletions in AZFc subregion cause spermatogenic defects.[24,25] Further, clinical studies have revealed that approximately 60% of deletion in the AZFc.[26] The AZFc subregion is about 3.5 Mb in size with repeated DNA amplicons that has made this subregion prone to structural variation in men.[27] Amplicons in AZFc are arranged in sequence families, where intrafamily sequence identity is above 99.9%, which makes them essential for structural rearrangement.[27] These amplicons contain genes required for spermatogenesis.

The functional role of amplicons/palindromes is not fully known. The AZFc subregion comprises two full palindromes - P1 and P2 along with the distal ends of P3 which accounts for approximately 90% of the sequence.[16] Repping *et al*. in 2006 established the AZFc genetic diversity in the Yq chromosome.[28] In later years, it was noticed that AZFc rearrangement is one of the important motifs for structural variation in the Y chromosome. In the AZFc subregion, evidence suggests that at the sequence level, both homologous- and nonhomologous-dependent pathways take place. There are many reports which support NAHR in the AZFc subregion.[29,30] Still, we have limited knowledge about the exact mechanism of NAHR at the sequence level. However, there are studies which showed activation of nonhomologous DNA end joining in AZFc.[31] Intrachromosomal homologous recombination is the major cause of genetic variability in AZFc. Lange *et al*. in 2009 have proposed the model for homologous recombination in palindromes where DNA double-strand break occurs within an amplicon which is frequent in male germ line.[32] The duplication in the AZFa subregion may disrupt the spermatogenesis process.[33] The studies associated with duplication analysis showed variation in outcome which may be due to the difference in geographical and ethnic population. The partial duplication was observed in oligozoospermia patients.[34] The duplication in the AZFc arises because of NAHR.[35]

Deleted in Azoospermia-A Candidate Gene

Deletion in the DAZ gene family is a most frequent event, which accounts for 13% of cases in men with infertility.[36] DAZ gene was obtained from the autosomal homologue, DAZ-like which is represented as red amplicon [Figure 1a] in the AZFc subregion with a high frequency of deletion in azoospermia men.[13,16] DAZ is present in multiple copies and organized in repeat cluster consist of four copies.[36] As the DAZ gene is expressed in almost all phases of germ cell development, it plays a variety of roles in the entire spermatogenesis process.[37] Deletion of the DAZ gene can involve many phenotypic variations from oligozoospermia to azoospermia as DAZ protein function in translation, control of meiosis, codes for RNA binding protein for germ cell, and maintain the primordial germ cell population.[16,19]

Partial Azoospermia Factor c Deletion

gr/gr deletion

A deletion of 1.6 Mb in Y chromosome is designated as gr/gr deletion, which was observed in infertile men with

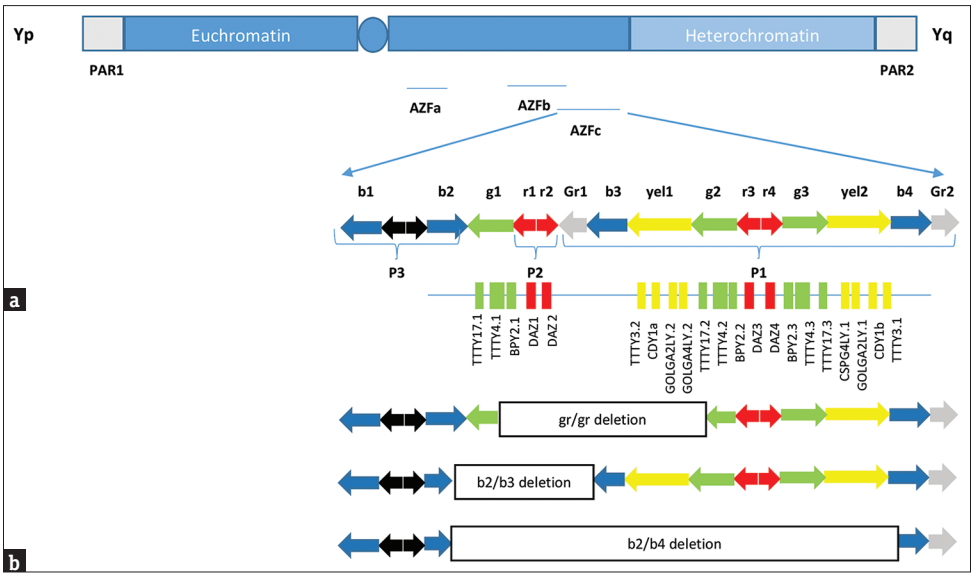

Figure 1: Layout of azoospermia factor c subregion of the human Y-chromosome. (a) The amplicon structure with color code; protein-coding and noncoding gene families in the azoospermia factor c subregion. (b) Schematic picture of gr/gr deletion, b1/b3 deletion, b2/b3 deletion, and b2/b4 deletion

spermatogenic failure.[38] Deletion of gr/gr removes part of the AZFc subregion consists of one copy of the CDY1 gene and two copies of DAZ gene along with other transcriptional units [Figure 1b].[36] It has been observed that the deletion in gr/gr and male infertility is dependent on ethnicity as well as a geographical region which should be considered as a risk factor in male with infertility.[34] Men with less sperm concentration, total sperm count, and total motility of sperm showed deletion for gr/gr when compared with individuals with no gr/gr deletion.[17] Gr/gr deletion arises due to the homologous recombination mechanism which is extensively studied in larger groups of fertile and infertile men in various parts of the world.[9]

b2/b3 deletion

The b2/b3 partial deletion in the AZFc subregion removes approximately 1.8 Mb, which is essential for spermatogenesis process.[39] Moreover, it has been observed that b2/b3 deletion is the bridge to genetic variability in the interval.[27] This deletion may occur due to gr/gr inversion or b2/b3 inversion.[15,40] It has also been reported in Chinese men about the association between b2/b3 partial deletions with infertility in men.[41] Whereas in many other studies, predisposition was not reported in an infertile population.[29,42]

b2/b4 deletion

b2/b4 deletion was reported first, which occurred due to recombination between b2 amplicon and b4 amplicon[16,19,43,44] [Figure 1b]. This deletion span about 3.5 Mb which eliminate whole AZFc subregion.[45] b2/b4 deletion is well characterized and is associated with spermatogenic impairment.[16,19] All the coding and noncoding gene families of the AZFc subregion were removed in b2/b4 deletion.[16,46]

Azoospermia Factor c Micro/Partial Deletions Screening using Different Approaches

If we consider all the genetic factors for male infertility, a study on microdeletions of the AZF region on the Y chromosome is of great importance as it has potential to transmit to the offspring. In previous studies by the European Academy of Andrology and the European Molecular Genetics Quality Network strongly recommended two sequence-tagged sites (STSs) for AZFc subregion (sY254 and sY255) which is specific to the DAZ gene in the P2 and P1 palindromes.[47,48] In the past few years, the partial deletions in the AZFc subregion have been described which is considered to play an important role in male fertility.[45,47-49] Deletion associated with b2/b4 pattern can be analyzed by sY160 STS marker.[16] The most widely used technique is simple STS-polymerase chain reaction (PCR), where a short sequence can be amplified using PCR. There are many studies where this technique is used for the detection of AZFc microdeletions.[47,48] The conventional PCR method amplifies one single template, to reduce the time, cost, and labor, multiplex-PCR was introduced to overcome the disadvantage of simple PCR, where two or more DNA templates can be amplified simultaneously in a single reaction. Therefore, multiplex-PCR is now widely used for genetic screening of Y chromosome microdeletions.[50] Commercial kits are available from different makers such as Diachem/Bird, Euroclone, Promega 2.0, Qiagen, etc.[49] Bunyan *et al.*

reported a method which is used for the detection of partial AZFc deletions using a Y chromosome-specific multiplex ligation-dependent probe amplification (MLPA) probemix (P360) known as MLPA assay.[51] A novel method known as universal primer-multiplex-PCR was adopted to overcome the disadvantage of conventional multiplex-PCR that is low specificity and sensitivity.[52] An alternative assay is the microassay technology, which has been introduced by Osborne *et al*. in 2007.[53] Hence, the improvement of the detection in relationship with Y chromosome microdeletions in various regions can help in identification of AZFc microdeletions in infertile men before undergoing assisted reproductive techniques and screening of oligozoospermic and azoospermic patients, sperm deposited in sperm bank, and population genetics research.

Conclusion

Yq deletions and/or mutations are considered as one of the most powerful causes of male infertility which affects the genes involved in spermatogenesis. Y chromosome infertility is inherited to future generations in Y-linked style. The high rates of deletion in the AZFc subregion suggest that it might devote a substantial number of deleterious new mutations. Examination for Y chromosome deletions in different ethnic groups helps to understand the deletion patterns and the negative effect on infertility rates.

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Conflicts of interest

There are no conflicts of interest.

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