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Instability of the Azoospermia Factors Locus on Y chromosome and Their Association with Male Infertility in Different Population

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Abstract
Y chromosome microdeletions are a mainly important reason of spermatogenetic impairment in males. Intrachromosomal recombination actions in large homologous repetitive sequence blocks are caused these microdeletions in spermatogenesis locus azoospermia factor (AZF). We obtained the literature by the PubMed and Google Scholar. The search keywords were azoospermia factors, microdeletions and male infertility. In this review, we selected the articles published in recent years about Y chromosome microdeletions that investigated their association with male infertility. The full texts of the appropriate articles were evaluated and a total of 82 articles were used. According to the results of reviewed articles, deletions in azoospermia regions eliminate one or more of the important genes and cause serious testiculopathy that are principal in male infertility. Our study indicated that Y chromosome microdeletions is variable in various population; these data propose that other genetic, epigenetic, nutritional and/or local factors are responsible for impairments in men fertility parameters observed in different populations.

Keywords: human Y chromosome, microdeletions, male infertility, azoospermia factors

Introduction
Infertility or sterility is generally described as the inability of a couple to conceive after one year of unprotected coitus (1). Male infertility is a frequent condition affecting up to 50% of infertility cases, which comprise 10-15% of couples (2,3). This form of infertility can be classified as genetic and non genetic disorder. Non genetic factors are; varicocele, structural abnormalities of the male genital tract, testicular maldescence, genital infections, chronic illness, exposure to chemicals and medication (4,5). Genetic factors involved in male infertility manifest as chromosomal disorders, mitochondrial DNA (mtDNA) mutations, monogenic disorders, multifactorial disorders and endocrine disorders of genetic origin (6). azoospermia and oligozoospermia in men might be due to mutations in the cystic fibrosis trans membrane conductance regulator (CFTR) gene (7), deletions of the azoospermic

factor region (AZF) of the Y chromosome (5) or structural and numerical abnormality of autosomes or the Y chromosome (8). Cytogenetic studies in infertile men have exposed a gene that controls spermatogenesis, localized on the long arm of the Y chromosome and designated as azoospermia factor (9). Deletion of these reigns is associated with azoospermia and oligozoospermia (5). These AZF genes code RNA binding proteins and may be involved in RNA metabolism, packaging and transport to cytoplasm, regulation of gene expression and RNA splicing (10).

Deletions in the Y chromosome are mostly de novo (11) and are believed to arise from recombination events between long stretches of highly repetitive DNA sequences during meiosis or early pre-implantation development (12). However, several cases of natural transmission of the microdeletions have been reported to date (13). These microdeletions of AZF are now recognized as the second most common genetic origin of spermatogenetic failure in infertile men (14).

In this review, we performed an extensive search on the PubMed and Google Scholar for the relevant articles that appeared in fifteen recent years and compared Y chromosome microdeletions possibly associated with male infertility in Iranian with other populations of the world. The full texts of the appropriate articles were evaluated and a total of 82 articles were reviewed.

Y chromosome and male infertility

Generally, of the 60 Mb length of the Y chromosome, 3 Mb belongs to pseudoautosomal regions and 57 Mb to a non recombining region that contains heterochromatic and euchromatic regions (15). Pseudoautosomal regions PAR1 and PAR2 (2.6 Mb, and 320 bp long, respectively) located on the two end part of the Y chromosome, are homolog with the termini of the X chromosome and are paired with the chromosome X during meiosis (16). A mosaic of heterochromatic and euchromatic sequences is the male specific region of the Y chromosome that include 95% of the Y chromosome length (17). Heterochromatin is located among palindromic motifs, gene families and repeated genes (18).

The euchromatic DNA sequences include approximately 23 Mb, that 8 Mb located on the short arm and 14.5 Mb on the long arm of the Y chromosome (16). Three classes of euchromatic sequences are recognized on the Y chromosome. Those may be transposed from the X chromosome during the evolution of the Y (X-transposed). Some of those extents like to sequence information from the X chromosome (X-degenerate) and those repetitive units across the closest short arm of the Yp and across most of the Yq (amplicons) (17). The sex-determining gene (SRY) is located in the X-degenerate region. The SRY gene expresses a transcription factor that direct the progress of male anatomical structures in the embryo (17,18).

The euchromatic region and some of the repeat-rich heterochromatic part of the Y chromosome are called “male-specific Y” (MSY). Male specific Y differentiates the sexes and comprises of 95% of the chromosome’s length (16). The MSY was known as NRY (non-recombinating region of the human Y chromosome) previously. It was believed that no recombining event occurred between the X and Y chromosomes during meiosis in this region. However, as MSY is actually flanked on both sides by pseudoautosomal regions, X-Y crossing over is a normal and frequent event during male meiosis (17).

From the male-specific Y reign, 18 different protein or 9 gene families have been recognized. The majority gate of testis-specific genes are present in multiple copies ranging

from one (TGIF2LY) to two (PRY, XKRY, HSFY, VCK) to three (BPY2) to four (DAZ, CDY) to six (RBMY) to nearly 35 (TSPY) on the Y chromosome. These genes exist in the closet and distal palindromic complexes surrounding the AZF region (20). At present, investigation of these deletions in infertile men is a standard clinical evaluation. Recently, other Y-chromosome structural variants, some of which affect gene copy number, have also been investigated (21).

AZF regions in Y chromosome

The AZF region is further sub divided into three non-overlapping hot spot regions defined as AZFa, AZFb and AZFc (5). In addition, recently AZFd region has been proposed between AZFb and AZFc. So far proximaly12 genes have been recognized from these regions (22).

The various Deletions in AZF regions have been associated with different phenotypes, such as AZFa region to Sertoli-cell-only syndrome (23), AZFb region to interruption in meiosis-I resulting in spermatogenic arrest (5) and AZFc region to hypospermatogenesis leading ultimately to severe oligospermia and azoospermia (24). Additional, AZFc and DAZ deletions are well known in the context of spermatogenic deficiency resulting in infertility (25). These AZF regions are screened and mapped molecularly for detection the possible deletions by using the conserved sequence tagged sites (STSs) (23).

The AZFa region

The AZFa is located at the distal portion of deletion interval 5 (subinterval 5C) and it has been estimated to span 400–600 kb of DNA (6). Complete deletion of AZFa is associated with azoospermia and no center of testicular spermatozoa (13,19). This region harbors 2 protein coding genes of USP9Y (ubiquity-specific protease9Y) and DBY (recently called DDX3Y) that are involved in deletions. They are both located in the X-degenerate region of euchromatin and have homologous genes on the X chromosome (15). USP9Y occupies less than semi of the AZFa interval, while the greater part of infertile males carrying AZFa deletions exhibit the absence of this entire interval (5). Already, USP9Y deletions have been identified that are compatible with sperm production and normal conception (13,26). Thus, published data emphasize to USP9Y not being essential for male fertility, as studied in other primate lineages where the gene inactivate (27). Nevertheless, it is still premature to discard the participation of USP9Y in the regulation of epistatic in male gametogenesis (28). The most recent data suggests that the AZFa phenotype requires more than one gene. They include DBY (DEAD box on the Y) and UTY (ubiquitous TPR motif on the Y). DBY consists of 17 exons (29) and encodes for a putative ATP-dependent RNA helicase, as it be a part of DEAD-box proteins (30). However, its special function in male germ cell development is still unknown (6). Primary studies on patients with only AZFa deletions, suggested that failure of USP9Y cause male infertility and the additional loss of DBY may create the phenotype inferior (31). Although DBY and USP9Y deletions only are not considered to be the main causes of infertility, but construed to be fine tuners of the normal spermatogenesis with other key players (13,26). AZFa deletions seem to repeatedly occur as a consequence of homologous recombination among two human endogenous retrovirus (HERV15) sequence blocks, separated by 800 kb (32).

The AZFb region

The AZFb extent from the distal part of deletion interval 5 to the nearly end of deletion interval 6 (subinterval 5O–6B) and it spans around 1–3 Mb of DNA (6). The AZFb deletion

is related with azoospermia and occurs mainly by a maturation arrest during the first meiotic prophase. In patients with AZFb deletion, the germ cells from mitotic (spermatogonia) and early meiotic stages (primary spermatocytes) remain plentiful in all tubules, but post-meiotic stages (spermatid) are rarely observed and constantly there is a complete deficiency of elongating spermatids and spermatozoa (33).

AZFb deletion is rare in the male infertile population, but this form is higher when limited criteria are used for select of patients (1-5%) (34). The known protein-encoding genes in this region that are associated with spermatogenesis are EIF1AY, RPS4Y2 and SMCY that are positioned in X-degenerate euchromatin. Also XKRY, PRY, HSFY and RBMY located in the ampliconic district (5). Two genes RBMY (RNA binding motif on the Y) and EIF1AY (eukaryotic translation initiation factor 1A, Y isoform) have been recognized in the AZFb region, so far (35). The RBMY was the first proposed gene responsible for AZFb deletions. A family of 20-50 genes and pseudogenes extend across both arms of the Y chromosome, including a group inside the AZFb region (35,36) and YRRM has been renamed as the "RBMY gene family" (37).The RBMY genes with a role in spermatogenesis are only expressed in the germline of the testis (spermatogonia, spermatocytes, and round spermatids) (35).

EIF1AY encodes an essential translation initiation factor that is not mainly AZFb-candidate gene. So far, no deletion removing this gene has been reported and its role in spermatogenesis is unknown (36). However, EIF1AY control abundant testis-specific transcripts in addition to ubiquitous transcripts (38), deletion of this gene may deregulate the RBMY1 expression leading to spermatogenic disruption (39) and suggesting that this gene may contribute to the AZFb phenotype (40).

The AZFc region

Molecular studies exhibited that AZFc is located at the proximal part of deletion interval 6 (subintervals 6C–6E) on the Y chromosome (9,40). The AZFc is a 4.5 Mb region of the euchromatin and its complete deletion play the most important role in male infertility (41). There are active copies of four protein-coding gene families' map to the AZFc interval: PRY2 (PTA-BL related Y), BPY2 (basic protein Y 2), DAZ (Deleted in Azoospermia) and CDY1 (Chromodomain Y 1), TTY2 (Testis transcript Y 2) (42). It is previously described; AZFc genes correspond to functional determinants of spermatogenesis, as evidenced by their germline-specific expression and by the fact that their deletion conveys phenotypical effects only for the gametogenic tissue (43,44).

Available information suggests that AZFc genes encode for germline-specific functions in: germ cell apoptosis (44), protein ubiquitination (45), transcriptional regulation coupled to chromatin remodeling (46) and storage, transport and translational activation of developmentally regulated transcripts (47). AZFc is one of the most commonly deletion in men with azoospermia or severe oligozoospermia (17,42). AZFc deletions are generated by intrachromosomal homologous recombination between repeated sequences blocks that called "amplicons" and organized in palindromic structures with identical sequences in each palindrome arm (48).

DAZ (Deletion in Azoospermia) is the most important candidate gene family in AZFc reign. DAZ is a part of a family of germ-cell-specific RNA binding proteins that are fundamental for gametogenesis (49). A two-gene cluster was duplicated and generated a two cluster/four-gene arrangement (DAZ1/2, DAZ3/4) 1.6 Mb within the AZFc region in Y

chromosome (50). DAZ gene has been transpose from chromosome 3 (3p25; DAZL1 locus) (51). The structure of the four copies is variable in exon, which is known as the "DAZ-repeat" (52). The DAZ gene family includes DAZL (DAZ-Like) and BOULE that are two autosomal homologs located on chromosome 3 and 2 respectively (53). The BOULE gene encodes an important factor of meiosis in male germ cells, regulating the expression of twine a cdc25 phosphatase, which promotes the development during meiosis (54). DAZL and DAZ act with the DAZAP1 protein (deleted in azoospermia associated protein 1) which alternates between the nucleus and the cytoplasm (55). AZFc deletions with remove the DAZ gene cluster led to a various spermatogenic alterations from azoospermia to oligozoospermia with different testicular phenotype (33).

Recently a partial deletion termed gr/gr was identified in the AZFc region as a risk factor for spermatogenic failure (56). This deletion removes two copies of the major AZFc candidate gene, DAZ (57). Also another deletion termed b2/b3 (1.8 Mb) which results in the absence of half the AZFc genes, seems do not have effect on fertility status and it is identified on a certain chromosome background commonly present in northern Eurasian populations (57,58).

The function of other genes of AZFc region (BPY2, CDY, TTY2 and PRY2) is unknown, but they have similar characteristics: there are multiple copies on the Y chromosome, expressed in the testes only, and they are specific for Y chromosome (38).

The AZFd region

Kent-First and coworkers described a fourth AZF region between the AZFb and AZFc termed the AZFd (59) which was related with mild oligospermia or abnormal sperm morphology (59,60). Until now, no candidate gene has been identified for this reign. However, deletion of the DYS237 locus in the AZFd region has frequently been identified and the genes located in this region indicated as a possibly important in spermatogenesis (60).

Many variations in AZFd deletion have been reported. STS markers SY133, SY145, SY153 and SY152 are suitable for screening the AZFd region. The microdeletion extends from SY153 in AZFd led to the junction of the euchromatic and heterochromatic regions (6).

STS based analysis

Investigations on the structural organization of the chromosome have advanced our understanding of Y chromosomal microdeletions. Large sets of primers covering palindromic complexes can be used for sequence-tagged site (STS)-based analysis of the genetic integrity on the Y chromosome (20). These analyses permit the detection of interstitial submicroscopic deletions that not visible at the cytogenetic analysis and can be identified only by STS-PCR or southern hybridization. These deletions are called microdeletions (40).

In recent studies the STS sites have proved to be repetitive sequences or polymorphic between individuals or races. Genomic DNA has a linear and adjacent sequence and STS is defined as the determination of their unique position inside the whole genome. However, after the completely verifying of genomic sequence, some of the original STSs were found to have repetitive or polymorphic sequences (21). Screening of such a large number of patient DNA samples with a various spectrum of Y chromosome anomalies is a laborious task (60) but today reliable STS markers on Y are available online (62).

Screening of the AZF region microdeletions

In the past few years, we have still noticeably limit knowledge of AZF genes function. Technical issues and the inherent complexity of this biological system can be considered as important reasons for this (63). The identification of the actual role of AZF candidate genes significantly advances our understanding of the spermatogenesis biology (6). The available evidence indicates that DDX3Y in AZFa, RBMY1A1 in AZFb, CDY and DAZ in AZFb/c represent key determining factors for spermatogenesis. Thus, progresses in this area are very importance for a more comprehensive outlook on the reproductive ability of the Y chromosome (63). In addition; an analysis of novel Y-chromosomal genes with a possible role in male germ cell development would explain other useful features of this important chromosome (40). Clearly, the future study lines in this field include the complete sequencing of AZF diversity across the Y chromosome in different population and of a more in-depth functional characterization of AZF genes. Properly, the finding specific deletions or other epigenetic alterations in AZF reign of infertile men may expose new clinically-relevant mutations. Combination of this information with functional data on the affected biological pathways would translate into significant conceptual advances in male reproductive genetics (63).

Results and Conclusion

Recent advances in molecular genetics have demonstrated that Y chromosome microdeletions are the mainly important reason of male infertility (64). AZF deletions are the euchromatic part of Y chromosome, which is dependable with spermatogenic disorders. Those deletions directly damage the genes located in this region and are responsible for the appropriate course of spermatogenesis (65). Therefore clearly microdeletions in these regions can be related with oligozoospermia and azoospermia (40). The AZFa and AZFc microdeletions were dependent with azoospermia in men (5). Deletions including AZFc and other reigns (AZFc+b+a, AZFc+b) are associated with entirety lack of testicular spermatozoa. AZFb and AZFa deletions are related with severe absence in spermatogenesis and Sertoli cell-only syndrome (64). Oligospermia can be associated with partial AZFb and total AZFc deletions in Y chromosome (66).

In the past decade, many research and clinical workplaces have reported screened microdeletions in infertile men and molecular diagnosis of this type of Y-chromosomal impairment has become a significant diagnostic test in laboratories worldwide dealing with this problem (14). It is indicated that the mainly common deletions occur in the AZFc and AZFb. Partial and complete deletions in AZFc are observed in 60% of the YCMs, and the 16% of AZF deletions in infertile males is the AZFb deletion site (67). In total, 35% of the deletions are AZFb, AZFbc or AZFabc (66) and only 2%to 5% of the deletions occur in the AZFa region (13,67).

In 1996, Vogt and colleagues preformed a study and screened 370 men with idiopathic azoospermia or severe oligospermia for submicroscopic deletions in the Yq. Thirteen of these men had microdeletions mapping to 3 different regions designated from proximal to distal as AZFa, AZFb, and AZFc (5). In a study, Hadjkacem-Loukiland and coworkers in Tunisia showed that between of 210 patients with azoospermia, oligozoospermia and

normozoospermia there were deletions of AZFc in 11%, AZFb in 7.3% and AZFa in 6.7% (69). In another study on 247 Saudi men with idiopathic azoospermia or oligospermia, 3.2% had YCM, consisted of 77% in the AZFc, 1.2% in the AZFb, and 1.2% in both AZFa and AZFc (70). Moreover, in USA, a total of 78 men with AZF deletions included 3.8% with AZFa deletion, 14% with AZFb and 54% with AZFc (33). In China, Y chromosome microdeletions were evaluated 78% AZFc deletions, 6.6% AZFb and 4.4% AZFa between of 45 infertile males (71).

According to the results of various studies of the different populations, AZFc region have a high frequency of microdeletions than to AZFb and AZFa. Also, several studies with similar results were performed on AZF regions microdeletions screening of infertile men in Iran.

In North western of Iran Omrani et al, of 99 patients with azoospermia or severe oligospermia men showed microdeletions in the AZF region. The deletions comprised the AZFc (87.5%) and AZFb (29.2%) regions (72). In another investigation at the Royan Infertility Clinic (Tehran-Iran), most Y microdeletions (95 of 185, 51.35 %) were found in the AZFc regions while AZFa regions were deleted in only 4 (2.16 %) and AZFb regions were deleted in only 8 cases (4.32 %). Also, combined deletions were detected in this study that included AZFac and AZFbc in 1 (0.54 %), and 29 (15.67 %) of the patients respectively (73).

These results of Iran are in agreement with studies of other countries and show that the AZFc regions microdeletions are more than AZFb,a in most infertile men of population. On the other hand, limited studies in the world exposed different results of AZF regions microdeletions.

For example a research in Southeast Turkey showed 1.3% microdeletions of the Y chromosome of a total of 80 infertile men (74). Also, Akbari Asbagh et al, from Iran reported only 5% AZFc deletion of a total 40 patients (75). The frequencies of detected AZF microdeletions in these studies were low and a value similar to that previously reported in the some studies (76-81). This may be one of the reasons for the failure to identify Y microdeletions in the infertile populations. The little size and slightly unbalanced nature of samples, ethnic differences, diverse patient selection criteria and methodological aspects, other additional epigenetic, genetic, local and nutritional factors can contribute to the dissimilarity between the different studies of various countries.

Also, there is a remarkable report of Iranian azoospermic infertile men that exposed the deletion in AZFb region was the most frequent (66.67%) followed by AZFc (41.67%), AZFd (33.33%) and AZFa (8.33%), respectively (82).

These differences in deletion frequency and localization between studies are owing to inclusion of different study groups or use of different STS sites primers or they may reflect genuine population variances and be correlated to a particular Y chromosome haplotype, genetic background or environmental influence. Finally, Y chromosome microdeletions cannot be predicted on the basis of clinical findings or even the results of semen analyses. Therefore the role of analyses of Y chromosome microdeletions in evaluating men with infertility remains to be determined.

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