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Gene Mutations Associated with Male Infertility

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1. Introduction

Infertility is a complex medical problem for several reasons. It is very frequent, since about 15% of couples worldwide fail to conceive, the male factor being involved in roughly 50% of cases. Secondly, the background of male infertility seems extremely heterogeneous including many environmental causes. Thirdly, in as many as 30% of individuals, the origin of infertility remains unknown (Poongothai et al., 2009). Chromosomal aberrations involving an abnormal number or structure of sex chromosomes or autosomes are found in approximately 5% of infertile men (for review see Ferlin et al., 2007). These aberrations often result in congenital syndromes, male infertility being one of their numerous features. Among them, Klinefelter syndrome is relatively common. It is caused by the presence of an extra X chromosome, 47,XXY. Another example is the 46,XX male syndrome. In a majority of cases, it results from translocation of a Y chromosome segment, containing the *SRY* gene, on the X chromosome. Structural aberrations of autosomes are much more frequent in males with isolated infertility than in the general population. For instance, Robertsonian translocation is 9-fold more frequent in infertile patients than in the general population. The most common Robertsonian translocation associated with male infertility is the one originating from chromosomes 13 and 14. Also reciprocal translocations are more frequent (4-10-fold) in infertile than in fertile males (for review see O'Flyn O'Brien et al., 2010). In the recent years much attention has been paid to mutations causing male infertility. These mutations were identified in genes known to be responsible for male germ cell development or, for other male reproductive processes. Thousands of genes in these categories are expressed in human testes and any of them can potentially cause infertility when mutated. This circumstance makes studies on genetic causes of male infertility extremely complex. Therefore, the generation of about 400 mouse models of male infertility in recent years has been very helpful to select the best human candidates for mutation screening in infertile men. These models represent defects at different steps of sperm cell development (Matzuk & Lamb, 2008). In fact, several hundred mutations have been identified in men suffering reproductive defects and by analogy with the mouse model, these mutations might affect human male reproduction. Also, state of the art technologies, such as genome-wide scanning, currently provide an enormous amount of new mutation records. Unexpectedly, this large amount of data on genetic variation contrasts with a very low number of reports describing well documented causative male infertility mutations. This is due to multiple obstacles in collecting additional necessary data. One of the major difficulties is

collection of DNA samples from some family members of the proband. This is hampered by the fact that in many instances infertility remains a very personal issue of the couple. This problem makes the study of inheritance patterns difficult. Secondly, the frequency of the mutated alleles is usually very low. Thirdly, the negative mutational effect is not obvious in many cases. The use of specific functional tests is necessary in such situations but often very difficult to establish. Therefore, simple testing for infertility causing mutations is so far limited to Y-chromosome microdeletions testing using commercially available PCR-based kits (for review see O'Flynn O'Brien et al., 2010). Studies on the gene content of the target AZF (Azoospermia Factor) region on the Y revealed few genes which encode nucleic acid binding proteins (for review see Navarro-Costa et al., 2010a). One of the first cloned and also the best studied among them is the *DAZ* (Deleted in Azoospermia) gene which contains an RNA-binding domain (Reijo et al., 1995). Studies of this gene in humans and in several model organisms from the fly to the mouse revealed that DAZ binds the 3'untranslated region (3'UTR) of specific mRNAs to regulate translation in germ cells (Fox et al., 2005). Later on, several other RNA-binding proteins cooperating with DAZ in 3'UTR mediated translational regulation in human male germ cells have been cloned and their status in infertile males was investigated. At the present time, even more attention has been given to the structure of 3'UTRs which are targets for several types of small regulatory RNAs (srRNAs). Importantly, these targets may not be properly recognized when mutated. This issue opens a new field in the research on male infertility and the underlying genetic causes.

2. Autosomal single-gene mutations causing non-syndromic male infertility

A small number of well documented cases of autosomal gene mutations causing non-syndromic male infertility are reviewed below. Several of theses genes cause a distinct spermatozoa defect in the semen of the patients when mutated. These autosomal gene mutations were identified in course of a candidate gene mutation screening strategy or by the genome-wide scanning approach.

2.1 *AURKC* gene mutations in macrozoospermia

The genome-wide microsatellite scanning was performed in 10 infertile males suffering from macrozoospermia and originating from North Africa. Identification of a homozygous frame-shift mutation c.114delC in *AURKC* (Aurora Kinase C) gene in all 10 patients provided a strong support for its causative effect and the macrozoospermia phenotype (Dieterich et al., 2007). This finding was reinforced by further studies showing that 100% (66 individuals) of infertile macrozoospermic men originating from North Africa carried an *AURKC* gene mutation in both alleles. Moreover, the majority of them were c.114delC homozygotes (Dieterich et al., 2007, 2009; Kerch et al., 2011). The remaining patients were c.114delC/c.436-2A→G or c.114delC/p.Cys229Tyr compound heterozygotes (Ben Khelifa et al.; 2011**,** Dieterich et al., 2009). Importantly, no homozygotes were found in the several control groups of fertile men (Dieterich et al., 2007, Kerch et al., 2011). Pedigree analysis in two families revealed that all homozygous males were infertile whereas homozygous females and heterozygous males were fertile (Dieterich et al., 2007, 2009). This indicates a recessive inheritance model for *AURKC* mutation transmission with the infertility phenotype restricted to men. The c.114delC mutation introduces a frameshift

p.Leu49TrpfsX22 resulting in premature stop codon. Indeed, a premature translational termination yielding a truncated protein lacking the kinase domain was demonstrated using a functional test (Dieterich et al., 2007). Spermatozoa of homozygous patients were all tetraploid indicating a cytokinesis arrest at the first meiotic division (Dieterich et al., 2009). Accordingly, the mutated *AURKC* gene has been associated with macrozoospermia characterized by a large-headed multiflagellar polyploid sperm. Also, mouse males characterized by *Aurkc* gene disruption presented with 20% of the sperm cells with characteristic macrozoospermic large heads (Kimmins et al., 2007). Consistent with this phenotype, this kinase was shown to localize at the centrosome suggesting a role in cell division (Kimura et al., 1999). Interestingly, although the *AURKC* gene mutations are deleterious only in male patients, Aurora Kinase C is highly expressed in both male (Bernard et al., 1998) and female gonads (Yan et al., 2005). These data indicate that, for couples with male infertility caused by *AURKC* gene mutation, the ISCI approach should not be encouraged.

2.2 *SPATA16* and *DPY19L2* gene mutations in globozoospermia

In course of infertility related genome-wide scan, *SPATA16* (spermatogenesis-associated 16) gene a mutation was identified in a consanguineous Ashkenazi Jewish family including three brothers suffering from globozoospermia. The same homozygous c.848G→A mutation of *SPATA16* gene was identified in all affected brothers. Since, both parents as well as two unaffected brothers were heterozygous and a healthy brother was a wild-type homozygote, an autosomal recessive inheritance of *SPATA16* gene mutation has been certified. Moreover, this mutation was absent in control group of fertile males. The functional analysis demonstrated that c.848G→A mutation altered the *SPATA16* pre-mRNA splicing process causing protein truncation. Namely, it disrupted the tetratricopeptide repeat (TRP) functional domain which is responsible for peptide-peptide interaction. The SPATA16 protein is specifically expressed in human testis and localizes to the Golgi apparatus (Xu et al., 2003). Accordingly, the mouse homologue localizes to the proacrosomic vesicles which at the spermatid stage are transported to the acrosome (Lu et al., 2006). While these data strongly suggested that *SPATA16* mutation caused globozoospermia, no causative *SPATA16* mutation was identified in additional 29 patients with the same phenotype (Dam et al., 2007).

Later on, two independent genome-wide scans of globozoospermic patients revealed a *DPY19L2* gene mutation underlying this phenotype. In the first study this mutation was found in a Jordanian consanguineous family including 10 siblings. In this family, four among five brothers suffering from complete globozoospermia, as well as three fertile brothers underwent the analysis. In all four analyzed infertile brothers a homozygous large ~200 kb deletion was identified. The only gene this deletion encompassed was the *DPY19L2*. Moreover, all fertile brothers were wild-type homozygotes at that *locus* (Koscinski et al., 2011). The second scan was performed on 20 patients mostly of the North African origin, suffering a complete globozoospermia. Also in this study a homozygous *DPY19L2* gene deletion was found in 15 out of 20 infertile men (Harbuz et al., 2011). Finally, a search for such *DPY19L2* deletions was performed on a third group of 28 globozoospermic patients. In this search, homozygous deletions were identified in 4 out of 28 infertile men. Differences of the deletion breakpoints identified in this group, suggested recurrent mutational events (Koscinski et al., 2011). A nonallelic homozygous recombination is the most probable bases for this mutational event,

given that the *DPY19L2* gene is surrounded by two low copy repeats (Harbuz et al., 2011; Koscinski et al., 2011). The *DPY19L2* locus represents a copy number variant (CNV), since duplications as well as heterozygous deletions were found with frequency 1:76 and 1:222, respectively, in the large almost 5 thousands group of healthy individuals. However, no homozygous *DPY19L2* gene deletion was observed in this group of people (Koscinski et al., 2011). The persuasive pedigree analysis, a high frequency of homozygous deletion encompassing *DPY19L2* gene in globozoospermic patients as well as the lack of homozygous deletions in the control group, altogether indicate that this gene is a globozoospermia factor essential for the sperm head elongation and acrosome forming. As expected, the *DPY19L2* gene is predominantly expressed in testis (Harbuz et al., 2011). The *C.elegans* homologue is involved in the cell polarity as it was shown in the human sperm cells (Honigberg et al., 2000).

2.3 *DNAI1*, *DNAH5* and *DNAH11* asthenozoospermia associated genes

Mutation screening of three dynein encoding genes *DNAI1*, *DNAH5* and *DNAH11*, was performed in a group of 91 asthenozoospermic Italian patients of Caucasian origin, including familial cases. A causative mutation was identified in each of these genes. One of them, a p.Arg663Cys amino acid substitution of *DNAI1* was found in two infertile brothers and two other unrelated patients. The p.Glu2666Asp mutation of the *DNAH5* gene, however, was present in only one man. Finally, the p.Ile3040Val nonsynonymous mutation of the *DNAH11* gene was identified in two first cousins and in one unrelated patient. In both familial cases, mutations were inherited from the mothers indicating an autosomal dominant pattern of dynein gene transmission with the infertility phenotype restricted to men. None of the three mutations were identified in the control group of 200 fertile men. The three mutations targeted the highly conserved amino acid sites and, moreover, were localized either within or near the functional dynein domain (Zuccarello et al., 2008). Taken together, the presence of familial asthenozoospermia cases associated with two dynein gene mutations, their location at highly conserved amino acid positions, as well as lack of all three dynein gene mutations in the control group, indicate that these mutations can be considered as asthenozoospermia causative mutations. The *DNAI1*, *DNAH5* and *DNAH11* genes were found to be expressed in testis and trachea (Bartoloni et al., 2002; Bush & Ferkol, 2006; Guichard et al., 2001; Hornef et al., 2006; Kispert et al., 2003; Noone et al., 2002; Olbrich et al., 2002; Pennarun et al., 1999; Schwabe et al., 2008; Zariwala et al., 2001, 2006, as cited in Zuccarello et al., 2008). The encoded proteins, axonemal dynein intermediate chain 1, axonemal dynein heavy chain 5 and axonemal dynein heavy chain 11 respectively, belong to the axonemal dynein cluster present in cilia and sperm tails. It was known that the presence of mutations in both alleles in a single dynein gene cause primary ciliary dyskinesia and Kartagener Syndrome which are usually associated with asthenozoospermia (for review see Escudier et al., 2009). Notably, this study shows that mutations in dynein encoding genes present in only one allele cause a non syndromic, male infertility phenotype.

2.4 *CATSPER1* gene mutations in oligo-astheno-theratozoospermia

The *CATSPER1(Cation channel sperm-associated protein 1)* gene was selected for mutation analysis after allelic homozygosity screening in two consanguineous Iranian families with familial cases of non-syndromic male infertility. Oligozoospermia, no motile sperm or sperm with lowered motility, but also increased counts of sperm with abnormal morphology were observed in the patients. In the first family, the homozygous insertion c.539-540insT was found

in two infertile brothers, whereas two other fertile brothers as well as the parents were heterozygous. In the second family, a different homozygous mutation, an insertion c.948-949insATGGC, was found in the infertile proband and his sister, the latter with unknown fertility status. None of these mutations were present in the control group of 579 fertile Italian men. Both mutations were frame shifts mutations resulting in premature stop codons, p.Lys180LysfsX9 and p.Asp317MetfsX18, respectively, producing a truncated protein lacking six transmembrane domains as well as the P loop (Avenarius et al., 2009). Therefore, in all probability, the CATSPER1 channel activity was abolished in homozygous patients. The *CATSPER1* gene was associated with oligo-astheno-theratozoospermia compound semen abnormality. The *CATSPER1* gene encodes the first of the four sperm-specific CATSPER voltage-gated calcium channels. Its mouse homologue is specifically expressed in the plasma membrane of the sperm tail element known as the principal piece (Ren et al., 2001) and it is required for calcium mediated hyperactivation of sperm motility (Carlson et al., 2003).

2.5 *NR5A1* (*SF-1*) azoospermia or oligozoospermia associated gene

Recently it has been shown, that mutations of *NR5A1* (nuclear receptor 5A1) gene, also known as *SF-1* (steroidogenic factor 1), may cause a non-syndromic spermatogenic failure. Mutation screening of the *NR5A1* gene in 315 mixed ancestry patients with azoospermia or oligozoospermia revealed 6 heterozygous mutations, 2 among them present in one allele, in 7 infertile men. A deleterious allele carrying a double mutation p.Gly123Ala/p.Pro129Leu was identified in three patients of 31, 37 and 42 years of age. While the youngest among them had a progressive loss of sperm cell concentration and quality, the two oldest patients suffered azoospermia. This may indicate that this double mutation at the heterozygous status causes a progressive degradation of sperm cells. The other four mutations, p.Pro311Leu, p.Arg191Cys, p.Gly121Ser and p.Asp238Asn, were identified in single patients and were associated with azoospermia or severe oligozoospermia phenotype. None of these mutations were observed in over 700 fertile or normozoospermic men from the mixed origin population nor in almost 1400 samples from numerous other worldwide populations (Bashamboo et al., 2010). Functional tests for the *NR5A1* mutations demonstrated that all of them abolished transactivation ability of SF-1 (Bashamboo et al., 2010, Laurenco et al., 2009). Although family pedigrees of the probands were not studied, the lack of these mutations in the vast control groups as well as the clear results of the functional tests altogether indicate that these *NR5A1* gene mutations are causative for infertility. The *NR5A1* gene encodes a transcriptional regulator playing a key role in many aspects of adrenal and reproductive development. The previously identified heterozygous mutations of this gene were associated with a wide spectrum of phenotypes including anorchia, gonadal dysgenesis 46,XY, genital ambiguity, micropenis, and cryptorchidism, as well as ovarian failure (for review see Lin & Achermann, 2008, Schimmer & White, 2010). It has been postulated that mutations which induce a more severe functional failure of the NR5A1 factor are associated with severe phenotypes while milder ones are associated with non syndromic infertility (Lin & Achermann, 2008).

3. The Y-chromosome and azoospermia factor region (AZF)

In 1976, a cytogenetic study performed on blood lymphocytes in a group of 1170 infertile men with azoospermia, revealed deletions of the long Y-chromosome arm (Yq) in six of them (Tiepolo & Zuffardi, 1976). This indicated the presence of a gene on the Yq, initially

called AZF for AZoospermia Factor, the lack of which have caused azoospermia phenotype. This initial finding has been followed by many reports describing smaller internal deletions within Yq. The deletion overlapping region has been named the azoospermia factor (AZF) region. Tremendous technological progress related to the Human Genome Project provided powerful molecular tools such as short Sequence Tagged Sites (STSs) to accurately delimit and precisely map single AZF deletions. This approach enabled identification of a large number of microdeletions in infertile men, mapping to different sites within AZF region. According to initial findings, specific phenotypes of spermatogenic failure were associated with distinct microdeletion locations within the AZF region. These associations served to designate three AZF subregions: AZFa, AZFb and AZFc (Figure 1) (Vogt et al., 1996). In the meantime, over 20 genes involved in spermatogenesis were identified in the AZF region (for review see Navarro-Costa et al., 2010a). Therefore, an attempt was made by many researchers to address whether there were correlations between definite infertility phenotypes and specific deleted AZF genes. The identification of such correlations could be beneficial for male infertility genetic diagnosis, as well as for Intra Cytoplasmic Sperm Injection (ICSI) prediction outcomes in infertile couples with male infertility factors.

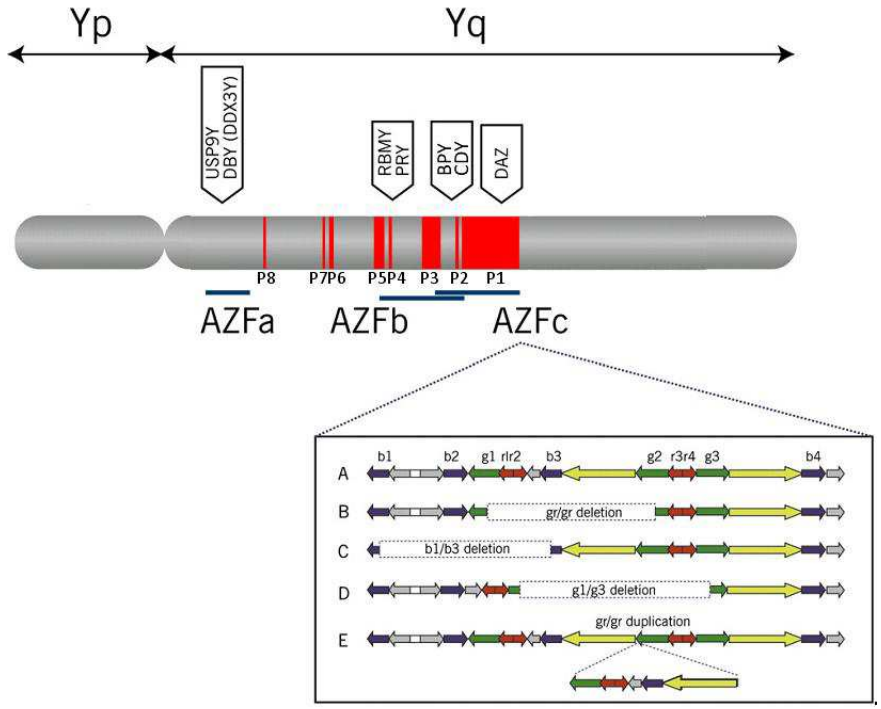

Fig. 1. The structure of AZF subregions and associated genes on the Y-chromosome. A, the ampliconic sequences mapped in the AZFc region, named after colors: blue (b), green (g), red (r), grey and yellow. B-D, the common types of subdeletions (gr/gr, b1/b3, g1/g3). E, a duplication (gr/gr) on the AZFc region. Genes representative of each subregion are indicated between two Y-chromosome schemes (modified O'Flynn O'Brien et al., 2010).

3.1 AZFa

This region is the most proximal AZF subregion. One of the genes it encodes is a single copy *DBY* gene, also called *DDX3Y*, with a corresponding copy on the X-chromosome. *DBY* encodes a Y-linked DEAD box RNA helicase of unknown function. Although this gene is ubiquitously transcribed, which is a hallmark of the Y-chromosome genes with a X-chromosome counterpart, DBY protein expression is limited to the premeiotic spermatogonia (Ditton et al., 2004). In 13 men with maturation arrest and 3 men with hypospermatogenesis, reduced levels of *DBY* transcripts in the testis was detected, while the other examined AZF genes were transcribed at the normal level (Lardone et al., 2007). This finding pointed out the importance of DBY for male reproduction. Furthermore, *DBY* gene deletions have been identified in 3 men with Sertoli Cell-Only Syndrome (SCOS), which is characterized by a lack of germ cells. However, these deletions removed both, the *DBY* as well as the closely mapping *USP9Y* gene (Sargent et al., 1999). For that reason, in this group of patients, the SCOS phenotype could not be assigned specifically to *DBY* gene. The second AZFa gene, *USP9Y*, encodes the Y-linked ubiquitin specific peptidase 9. It is the only gene reported as an isolated deleted gene of AZF region associated with infertility. It has been later shown, however, that a loss or partial deletion of *USP9Y* gene may cause variable phenotypes from azoospermia (Sun et al., 1999), through oligozoospermia (Brown et al., 1998) up to subfertility (Krausz et al., 2006). It seems that the phenotype associated with the *USP9Y* gene defects varies according to the specific carriers or some other factors which are still unknown. Moreover, a dysfunctional *USP9Y* gene was reported to be passed to the next generation in one family (Luddi et al., 2009). Thus, it seems that the *DBY* gene plays a more crucial role in male reproduction than the *USP9Y* gene.

3.2 AZFb

The *RBMY* gene was the first candidate gene from the AZFb subregion, proposed to be involved in spermatogenesis (Ma et al., 1993). One of the six copies of this gene located on the Y chromosome, *RBMY1*, encodes a Y-linked RNA binding motif protein, which is a testis-specific splicing factor expressed in the nuclei of male germ cells (for review see Vogt, 2005). One man manifesting azoospermia and the maturation arrest phenotype, reduction of *RBMY1* expression was demonstrated. Given that an AZFc deletion was also present, the significance of the *RBMY1* expression reduction could not be assessed (Lavery et al., 2007). However, severe spermatogenic failure and SCOS or hypospermatogenesis was also reported in men carrying microdeletions removing various genes of AZFb area, excluding the *RBMY* gene (Ferlin et al., 2003). This might suggest that some additional AZFb genes contribute to a similar histological and clinical infertility phenotype when deleted. The strongest candidate is a family of *PRY* genes which are involved in the regulation of apoptosis during the spermatogenic process (Stouffs et at., 2004). Furthermore, spermatogenesis was completely arrested before or at the meiosis stage, when both genes were removed (Vogt et al., 1996). This suggests that, in the AZFb region, the *RBMY* and *PRY* genes are crucial for the male reproduction.

3.3 AZFc

This subregion encodes several protein-coding gene families, including three copies of *BPY* and two copies of the *CDY* gene. However, the functions of the *BPY* and *CDY* genes are not

known yet (Kuroda-Kawaguchi et al., 2001). This subregion encodes four copies of the *DAZ* (Deleted in AZoospermia) gene, one of the first cloned AZF genes, encoding a germ cell–specific RNA binding protein (Reijo et al., 1995). Deletions of *DAZ2*, *DAZ3*, or *DAZ4* were identified in infertile patients but also in fertile men. In the latter case they were considered familial variants transmitted from father to son (Vogt et al., 1996; Saxena et al., 2000; Fernandes et al., 2002, 2004). Only, *DAZ1*/*DAZ2* double deletions were reported to be restricted to infertile men (Fernandes et al., 2006). It suggests that expression of *DAZ1* is crucial for spermatogenesis (Fernandes et al., 2002), although a case of one fertile man carrying a *DAZ1* deletion has been reported (Machev et al., 2004).

It has been recently demonstrated that genes related to the AZFb and AZFc subregions are located in so-called "amplicons". They represent subregions containing a series of inversely repeated units organized in eight palindromes, P8-P1, as ordered from the most proximal up to the most terminal towards the centromere (Figure 1). A peculiarity of amplicons compared to other AZF regions is their high density of the Y-chromosome genes with testis predominant expression (Kuroda-Kawaguchi et al., 2001; Skaletsky et al., 2003). Moreover, it has been shown that AZFb plus the AZFc subregion contain large segments of duplicated sequence with the proximal end of the AZFc overlapping with the distal end of AZFb (Figure 1) (Repping et al., 2002). Although complete AZFc deletions, removing 3.5 Mb between the b2/b4 amplicons, are most commonly found, a number of smaller, partial AZFc subdeletions have also been identified (Figure 1B-D) (for review see Navarro-Costa et al., 2010b). These partial deletions were associated with variable semen phenotypes, ranging from normospermic to azoospermic. This phenotypic diversity has been suggested to be a consequence of different origins, that undergo differential evolution over generations within distinct ethnic groups, reflecting specific environmental pressures (Bateson et al., 2004). This was observed in the cases of the most frequent deletions of the AZFc region, the gr/gr subdeletions (Figure 1B). Gr/gr subdeletions removing a 1.6 Mb fragment of AZFc region were identified as risk factors for spermatogenic failure in several studies, while in others such an association was not found (for review see Navarro-Costa et al., 2010b). Surprisingly, an analysis of the Han Chinese population revealed that a duplication of the gr/gr region (Figure 1E) may be deleterious for fertility (Lin et al.; 2007). Given that, the gr/gr deletions can be passed from father to son, the gr/gr deletion results in subfertility rather than in complete infertility (Poongothai et al., 2009). This picture appears even more complex due to some other studies indicating no association between spermatogenesis and the genes in the AZFc region (Saut et al., 2002).

3.4 Genotype-phenotype correlations in infertile males carrying AZF region microdeletions

Microdeletions of AZFa subregion are relatively rare. They are responsible for infertility in 1% of men with NonObstructive Azoospermia (NOA). In the majority of cases, patients with the AZFa deletions lack germ cells in seminiferous tubules or show the presence of germ cells only in the minority of tubules. Therefore, it is assumed that AZFa microdeletions correlate with SCOS phenotype (for review see Sadeghi-Nejad & Farrokhi, 2007). Microdeletions in AZFb are responsible for infertility in 1-2% of men with NOA. However, in contrast to AZFa, patients carrying AZFb deletions present with variable phenotypes. Namely, in about half of such cases, a maturation arrest phenotype at the stage of primary spermatocytes was found (for review see Vogt, 2005). Moreover, patients carrying large

AZFb deletions are azoospermic whereas those harboring smaller AZFb deletions present with a range of infertile phenotypes, including severe or even mild oligozoospermia. The most common aberrations, however, which occur in AZF are multiple gene deletions encompassing both, AZFb and AZFc subregions, resulting in a wide range of infertility phenotypes (for review see Navarro-Costa et al., 2010a). Also AZFc deletions are responsible for a variety of phenotypes ranging from azoospermia to severe oligozoospermia. In addition, AZFc deletions are the most frequently identified microdeletions in patients with NOA. They are responsible for up to 12% of azoospermia and 6% of severe oligozoospermia cases (Kuroda-Kawaguchi et al., 2001).

Today the Y chromosome microdeletions are the most frequently known genetic cause of severe spermatogenic impairment. Therefore, it is important for men suffering azoospermia or severe oligozoospermia to undergo tests for AZF microdeletions. Importantly, the only molecular genetic tests which are routinely performed to identify the cause of male infertility are those for microdeletion identification within the AZF region. These tests are PCR-based, find the most commonly deleted STSs and are commercially available. They are of great importance for both, a correct diagnosis, as well as for genetic counseling. Knowledge about the type of AZF deletion may help the clinician to select patients for, and to determine the best type of, Artificial Reproductive Technology (ART). It is known that sometimes such men can still father children with help of ART, including ICSI. Moreover, the combination of ICSI with testicular sperm recovery offers even azoospermic men the possibility of fathering their own genetic children. However, men carrying AZFc microdeletions must realize that their male offspring will almost certainly be subfertile (for review see Poongothai et al., 2009).

4. 3'UTR-mediated translational regulation and male infertility

Identification of *DAZ*, the first AZFc gene causing male infertility, stimulated much research on its role in human germ cell development as well as in model organisms such as flies, worms, frogs and the mouse. Nowadays, *DAZ* is the best studied among other so far weakly assessed AZF genes. Four Y-chromosome copies of *DAZ* arose most likely by amplification of the original autosomal *DAZL* (*DAZ*-Like) gene (Saxena et al., 1996). The DAZ protein family which emerged from this genomic reshuffling is thought to control meiosis and maintenance of germ cells (Reynolds et al., 2005). The DAZ proteins contain an RNA-binding domain suggesting involvement in RNA regulation (Saxena et al., 1996). In particular, DAZ was later shown to bind another highly conserved RNA-binding protein, PUMILIO2 (Moore et al., 2003). This protein was earlier identified as a translational repressor in body patterning and germ cell development of the fly (Wharton et al., 1998). Interaction with PUMILIO2 highlights involvement of DAZ in a type translational regulation mediated by the 3'UTR, which is crucial in many developmental processes, including that of germ cells. This regulation involves recognition of specific nucleotide motifs within 3'UTRs by definite complexes of RNA-binding proteins. By this means, specific mRNAs are stimulated for translation or are directed towards P-bodies, cytoplasmic storage and degradation centres (for review see Kishore et al., 2010). Therefore, one can imagine that elimination of PUMILIO2–binding partner caused by *DAZ* gene deletions may bring about translational deregulation of specific mRNAs in the male germ cells resulting in male infertility. An unsuccessful attempt has been made to identify *PUMILIO2* gene

mutations that potentially could disable DAZ-PUMILIO2 interaction, causing infertility phenotypes similar to those found with DAZ deletions (Kusz et al., 2006). Two other PUMILIO2-binding, male germ cell specific, highly conserved RNA-binding proteins, NANOS2 and NANOS3, were not found to be dysfunctional in genetic screens for mutations in a series of infertile males (Kusz et al., 2009a; Kusz et al., 2009b). Inactivation of both genes (Nanos2 and Nanos3) cause male infertility in the mouse (Tsuda et al., 2003).

The GEMIN3, a miRNA biogenesis factor (Mourelatos et al., 2002) has recently been found to bind PUMILIO2 and NANOS1 proteins within the chromatoid body, a structure analogous to P-bodies of somatic cells (Ginter-Matuszewska et al., 2011). This finding strongly suggests participation of miRNAs in 3'UTR mediated translational regulation in partnership with these proteins in human male germ cells. Many recently identified potential PUMILIO2 mRNA targets contain several predicted miRNA binding sites (Galgano et al., 2008). Moreover, various microRNAs as well as other small regulatory RNAs are expressed in human male germ cells and they seem to be active in spermatogenesis (He et al., 2009). Their biogenesis and involvement in male infertility is summarized bellow.

5. Small regulatory RNAs in reproduction and male infertility

There is a growing body of data indicating that one of the most important class of regulatory noncoding RNAs discovered in recent years, the small regulatory RNAs (srRNAs), play a major role in human spermatogenesis and may contribute to male infertility. Most of these RNAs are involved in RNA interference (RNAi), a phenomenon which usually leads to gene silencing. The length of srRNAs ranges between 20-30 nt. srRNAs contain 5' phosphate and 3'-OH groups which can be modified. All of them are loaded into specific effector complexes named RNA Induced Silencing Complexes (RISCs) to silence specific RNA targets (for review see Czech & Hannon, 2011). There are three major classes of srRNAs: microRNAs (miRNAs), short interfering RNAs (siRNAs) and PIWI interacting RNAs (piRNAs).

5.1 miRNAs

The first miRNA was discovered in *C. elegans*, when it was found that the *lin-4* gene, known for its role in early larval development timing, encodes two short RNAs, one ~22 nt and the second one ~61 nt. The longer molecule, which folds into a stem-loop structure, turned out to be a precursor of the shorter one. Interestingly, both molecules were complementary to multiple sites present in 3' UnTranslated region (3'UTR) of the *lin-14* mRNA. This enables *lin-4* RNAs to bind the *lin-14* 3'-UTR, which results in translational repression of *lin-14* mRNA (Lee et al., 1993). Seven years later, a second 22 nt regulatory *let-7* miRNA encoded in l*et-7* gene was identified. This miRNA promotes later larval development timing in the *C. elegans* (Reinhart et al., 2000). Soon after, *let-7* homologues, as well as a large number of other miRNAs, were discovered in the fly, many other animal groups and the human genome (for review see Bartel, 2004). Among over one thousand miRNAs identified in humans, some are ubiquitous whereas some other are tissue specific, e.g., are only expressed in testis (e.g. Bentwich et al., 2005).

The primary miRNA precursors known as pri-miRNAs are transcribed usually by RNA polymerase II, or in some cases RNA polymerase III. All known human pri-miRNAs contain

a 5'cap structure and a 3'polyA tail (Cai et al., 2004). These miRNA precursors are cleaved by an RNase III named Drosha, resulting in 60-80 nt pre-miRNA molecules containing a hairpin loop with two 3' overhanging nucleotides. The human Drosha enzyme contains four distinct domains: catalytic, double-stranded RNA binding, proline-rich, and serine and arginine-rich domains. Drosha is a component of a 500 kDa miRNA processing complex. Another crucial component interacting with Drosha and necessary for pre-miRNA processing in humans is the DGCR8 protein (DiGeorge syndrome critical region gene 8) (Gregory et al., 2004)

The next step in miRNA biogenesis is the export of pre-miRNA from the nucleus to the cytoplasm. This process is mediated by Exportin 5 protein, a member of cariopherin family. The loading of cargo on Exportin 5 requires binding of the phosphorylated Ran-GTP, a member of GTPases family. Hydrolysis of Ran-GTP to Ran-GDP causes cargo release. Binding of Exportin 5 to pre-miRNA requires the presence of a minihelix motif with a stem built of fourteen base pairs at the 5' end and 3-8 overhanging nucleotides at the 3' end (for review see Kim, 2004). Once in the cytoplasm, pre-miRNAs are cleaved by Dicer, a type of RNase III which cuts the last base pair of the stem-loop. The double stranded cleavage product contains 5' phosphate and two 3' overhanging nucleotides. To perform this activity in humans, Dicer cooperates with TRPB and PACT proteins (Kok et al., 2007).

After this maturation step, the miRNA-miRNA* duplex (miRNA-guide strand and miRNA*-passenger strand) are loaded into the RISC complex which contains Argonaute (AGO) protein, a member of Argonaute superfamily. Among human ARGONAUTE proteins (AGO1-4) only AGO2 has slicer activity (Parker & Barford, 2006; Wang et al., 2009). Generally the miRNA strand characterized by a less thermodynamically stable 5' end of the miRNA duplex is selected as the guide strand within miRISC complex (Schwarz et al., 2003). It has been proposed that the passenger strand is removed in the cleavage-independent manner by unwinding (Kawamata et al., 2009; Yoda et al. 2010). The guide strand is responsible for miRISC specific mRNA recognition. The miRISC binds to recognition sequences located within 3'UTR of mRNA complementary to the guide strand, although this complementarity is usually not complete. This binding induces translational repression or, rarely, stimulation through a mechanism which is not fully understood (for review see Bartel, 2009; Huntzinger & Izzauralde, 2011). Four alternative ways of translational repression have been proposed: inhibition at the translation initiation or elongation, co-transcriptional protein degradation or premature translational termination. Intensive studies are in progress to decipher this intricate, miRNA mediated, posttranscriptional silencing mechanism (for review see Djuranovic et al., 2011; Huntzinger & Izzauralde, 2011). Meanwhile, it has been demonstrated that single nucleotide polymorphisms (SNPs) identified at various miRNA binding sites in 3'UTRs of specific mRNAs may cause diseases, including human male infertility.

The miRNA expression profile analysis using miRCURY™ LNA microarray platform was performed in testis samples originating from 3 patients diagnosed with non-obstructive azoospermia (NOA). These profiles were compared with profiles from testis samples of two fertile individuals who underwent orchiectomy due to prostate carcinoma. By this approach a total of 173 miRNAs were selected to be differentially expressed in NOA patients compared to fertile controls. 19 of them were upregulated whereas 154 downregulated (Lian et al., 2009). Among 154 downregulated miRNAs, at least 12 (7.8%) were found to be testis

specific in the mouse (Ro et al., 2007). The results obtained by microarray analysis were confirmed by real time RT-PCR, on a selected group of four miRNAs: *miR-302a*, *miR-491-3p*, *miR-520d-3p* and *miR-383*. The first two *miR-302a*, *miR-491-3p* were upregulated, while *miR-520d-3p* and *miR-383* were downregulated in NOA patients. Additionally, downregulation of *miR-383* in these patients was confirmed by *in situ* hybridization. Interestingly, specific expression profiles were found to be correlated with miRNA genomic cluster localization. While chromosomes 14, 19 and X appeared to encode a significant number (62) of downregulated miRNA genes from this study, the majority of upregulated miRNA genes mapped to chromosome 17. For example, the expression of 12 out of 13 members of a miRNA cluster on human chromosome 14q32.31 were downregulated in infertile men, with only one, the *miR-654-5p*, upregulated. An opposite bias was found in two miRNA gene clusters located on chromosome 19q13.42. However, in one of these clusters only two miRNAs, *mir-371* and *mir-372* were downregulated, the *mir-373-5p* of the same cluster being upregulated. The second cluster contains 13 nonconserved downregulated miRNAs. A bioinformatic analysis was performed to select for potential targets of these differentially expressed miRNAs. The identified potential target genes, *TIMP3*, *SOX9* and *GAD45G* (Lian et al., 2009), had been previously shown to be upregulated in testis of infertile patients (Rockett et al., 2004). The *TIMP3* gene, is a potential target for downregulated *miR-1*, *miR-181a*, *miR-221* and *miR-9**. This gene is involved in testis development and differentiation (Zeng et al, 1998). The second target encoding a well described transcription factor *SOX9*, is probably regulated by *mir-145* (Lian et al., 2009) and is required for testis determination and development but also for spermatogenesis (Schumacher et al, 2008). Finally, the third target gene encoding GAD45G, an apoptosis inducer and cell growth inhibitor in response to stress shock (Ying et al., 2005), is also a potential target of *miR*-383 (Lian et al., 2009). It is expected that expression of those three target genes might significantly contribute to male infertility. In addition, several members of the *mir-17-92* and *mir-371,2,3* clusters which are downregulated in NOA patients testicular tissue, are considered potential novel oncogenes due to their participation in the development of human testicular germ cell tumors (TGCTs). For example, the mir-17-92 cluster, which is rarely expressed in NOA patients, is highly expressed in the carcinoma in situ (CIS) testis tumor. These miRNAs act as apoptosis inhibitors by means of translational downregulation of E2F transcription factor 1(E2F1) in CIS cells. Therefore, the lowered expression of these miRNAs observed in NOA patients may contribute to increased apoptosis in the gonadal tissue (Lin et al., 1999, Novotny et al., 2007 Voorhoeve et al., 2006). Molecular consequences of *miR-383* downregulation were further investigated. Consistent with the miRNA microarray results the expression of *miR-383* was significantly decreased in testicular specimens of the patients with maturation arrest (MA). In addition, real-time PCR results also revealed a significant downregulation of miR-383 expression in testes obtained from NOA patients compared with normal controls, as previously reported (Lian et al., 2009). This downregulation may be exclusive for MA patients, as *miR-383* was not altered in infertile patients with hypospermatogenesis (HA). Regulation of *miR-383* expression takes place mainly at the transcription level, although additional layers of post-transcriptional regulation exist. The testicular embryonal carcinoma cell line (NT2) which shows overexpression of *miR-383* manifests with significantly decelerated cell proliferation, G1 cell cycle arrest and apoptosis stimulation. Inhibition of endogenous *miR-383* expression resulted in a significant stimulation of proliferation, a decrease of cell number in the G1 phase, and suppression of basal levels of

apoptosis. Interferon regulatory factor-1 (IRF1) was predicted and experimentally verified as being the *miR-383* target gene. Since the effects of IRF1 silencing in NT2 cells were very similar to the ones caused by *miR-383* overexpression, this may suggest a promitogenic role of *miR-383* in NT2 cells. This effect is opposite to that found in most other cell lines from other tissues (for review see Kroger et al., 2002). The negative correlation between *miR-383* and IRF1 expression was confirmed *in vivo* in the mouse testes. It was further investigated since IRF1 regulates a set of genes involved in apoptosis, cell cycle and DNA repair. The mRNA and protein expression level of the cell cycle *Cyclin D1*, *CDK2* and *p21* genes was positively correlated with IRF1 levels in NT2 cells. Moreover, silencing of Cyclin D1 resulted in inhibition of proliferation due to arrest at the G1 phase. Silencing of Cyclin D1 caused a dramatic enhancement of the *miR-383* effects. Meanwhile, silencing of p21 caused partial inhibition of *miR-383*-induced G1-phase arrest. It was reported that Cyclin D and p21 in complex with CDK4 are required for phosphorylated retinoblastoma protein (pRb)-mediated G1/S transition. This phosphorylation was inhibited by *miR-383* which also repressed CDK4 expression by the proteasome-dependent pathway. Immunostaining of cyclin D1, p21, CDK2, CDK4 and phosphorylated pRb (P-pRb) in testis tissue of MA patients revealed higher expression of all these proteins in some seminiferous tubules. However, in the MA patients studied, enhanced nuclear staining for P-pRb was the only finding present in all of them. These results indicate that dysregulation of the *miR-383*-mediated pRb pathways might contribute to spermatogenic failure in MA patients. Interestingly, a loss of pRb expression is often associated with occurrence of testis germ cell tumor (TGCT). The existence of the widespread expression of P-pRb in the testes of MA patients indicates a status of "physiological absence of pRb" in infertile men. TGCTs were reported to occur three times more frequently in infertile than fertile man. Therefore, activated P-pRb expression caused by *miR-383* downregulation could be associated with a higher frequency of TGCTs (Lian et al., 2010).

In a recent bioinformatic study of 140 mRNAs involved in mammalian spermatogenesis (database founded), 21 human mRNAs carried, in total, 39 SNPs which occurred in the predicted miRNA-binding sites. Six among those SNPs were demonstrated to induce a significant change of Gibbs binding free energy value at a rate higher than 5.27 kJ/mol. This alteration indicated a possible disabling of miRNA interaction. These potentially causative six SNPs were located in the 3'UTRs of five mRNAs encoding CYP19, Serpina5, CGA, CPEB1 and CPEB2 spermatogenic proteins. As the next step, a total number of 494 patients manifesting with azoospermia or severe oligozoospermia and 357 fertile males were tested for these 6 SNPs. One of them, a mutant allele rs2303846 identified in *CPEB1* gene, was correlated with infertility. Also the mutant allele rs6631 (A→T transversion) of the *CGA* gene was found to be associated with idiopathic male infertility. The following miRNAs were predicted to bind the site containing the rs6631 SNP in *CGA*: *miR-610*, *miR-34c-5p* and *miR-1302*. While the *miR-1302* was predicted to bind more stably the rs6631-A allele than rs6631-T, a reverse correlation was found for *miR-610* and *miR-34c-5p*. To confirm these predictions, dual-luciferase reporter assays were performed in human HEK293T cells. At the beginning, these cells were tested for expression of these three miRNAs. As the next step, the cells were transfected with constructs containing a luciferase reporter cDNA in fusion with the modified *CGA* 3'UTR representing rs6631-A or rs6631-T alleles, or with a deleted miRNA binding site. The *miR-1302* was found to negatively regulate *CGA* expression given that rs6631 related A→T substitution disrupted the repression by 26% (H. Zhang et al.,

2011). Possibly the resulting *CGA* overexpression is the direct cause of idiopathic male infertility. The *CGA* gene encodes the α-subunit of glycoprotein hormones, such as thyrotropin (the pituitary TSH), lutropin (LH), follitropin (FSH), and the placental chorion gonadotropin (human chorionic gonadotropin, hCG). These hormones play important roles in thyroid and in gonadal development and function (Baenziger & Green, 1988). Heterodimerization of α- and β-subunits is a critical event for their function (Bieche et al., 2002). Disruption of CGA expression causes a lack of biologically active FSH, LH and TSH hormones and results in hypogonadism. The α-subunit alone has some growth factor activity and it can induce lactotrope differentiation and the subsequent secretion of prolactin (PRL) (Begeot et al., 1984; Blithe et al., 1991; Lapthorn et al., 1994; Kendall et al., 1995). While CGA and PRL are known to play important roles in gonadal development, one can assume that overexpression of CGA may elevate the risk of male infertility (H. Zhang et al., 2011).

In a separate study on human 5 day embryos (blastocyst stage) originating from fertile donors, or from male factor infertility couples, 12 miRNAs (*RNU48*, *let-7a*, *let-7b*, *let-7c*, *let-7g*, *miR-19a*, *miR-19b*, *miR-21*, *miR-24*, *miR-34b*, *miR-92*, and *miR-93*) were selected because of their expression at that developmental stage. Interestingly, a significant decrease in expression of *let-7a* and *miR-24* was observed in blastocysts derived from couples with a male factor infertility compared to fertile donors. This decrease was associated with significantly altered expression of two genes. One of these genes encodes a decay promoting factor, KHSRP, while the second one encodes a transcription factor, NFAT5. The corresponding mRNAs were predicted targets for *miR-24*. While the decrease of *miR-24* in male infertility factor blastocysts was correlated with a significant increase of KHSRP expression, the expression of NFAT5 was decreased. The gene ontology (GO) biological processes annotation for the *let-7a* and *miR-24* targets revealed involvement in cell growth and maintenance, transcription and protein metabolism. The GO molecular function annotation indicates signal transducer and nucleic acid binding activity (McCallie et al., 2010). In addition, lowered or lack of expression of *let-7a* and *miR-24* was previously found to be associated with several types of human neoplasia. Malignant melanoma, gastric carcinoma, lymphoma and chronic lymphatic leukemia were associated with a lack of *let-7a* expression (Marton et al., 2008; Muller & Bosserhoff, 2008; Sampson et al., 2007; H. H. Zhang et al., 2007). Likewise, *miR-24* was shown to be responsible for downregulation of a tumor suppressor gene *p16* in cervical carcinoma cells a decreased level of *miR-24* caused increase of *p16* expression resulting in replicative senescence (Lal et al., 2008). These findings may suggest a possible role for embryonic miRNAs in etiology of human male infertility and several cancers (McCallie et al., 2010).

5.2 siRNAs

The siRNAs were first discovered in plants (Hamilton & Baulcombe, 1999). Although, in the animal kingdom, biogenesis of siRNA is best understood in *D. melanogaster*, this process seems to be very similar in mammals. siRNAs are derived from long dsRNAs. These long precursors are processed to siRNA duplexes by Dicer 2 (DCR2) (Lee et al., 2004). In mammals, Dicer is also responsible for siRNA and miRNA processing (for review see Jaskiewicz & Filipowicz, 2008). The siRNAs are processed from single stranded transcripts that can form long hairpins or dsRNAs derived from paired single stranded molecules. The siRNA duplexes are incorporated into RISC (siRISC). During siRISC assembly, the

passenger strand of siRNA is cleaved by the AGO protein which has a slicer activity (AGO2 in human) (for review see Czech and Hannon, 2011). The mature siRISC binds complementary mRNA to form an effector complex. The siRNA guides AGO2 to catalyze cleavage of the target mRNA at a site that is ~10 nt distant from the 5' of siRNA. After AGO2 cleavage, the target mRNA is degraded and the RISC is recycled to cleave the next mRNA target molecules (Elbashir, et al., 2001; Liu. et al., 2004; Meister et al., 2004; Song et al., 2004). The siRNA mechanism functions in mammalian reproduction, since endogenous siRNAs were found to be in abundant in mouse oocytes, ES cells and spermatogenic cells (Babiarz et al., 2008; Song et al., 2011 Tam et al., 2008; Watanabe et al., 2008). Involvement of siRNA phenomena in male human infertility is a matter of future studies.

5.3 piRNAs

Piwi-interacting RNAs (piRNAs) are a class of small regulatory RNAs of the 24-30 nt. These srRNAs differ from miRNAs and siRNAs in that they are not produced by Dicer enzymes and do not require formation of a stable double-stranded RNA intermediates for their biogenesis (for review see Simonelig, 2011). piRNAs also vary from other srRNAs in that they were described primarily in gonads in many animal species from *Drosophila* to mammals. The principal role of piRNAs is to protect the genome against transposable elements (TEs) which represent as much as 45% of human DNA and which are known to move and replicate within the genome (Lander et al. 2001). Derepression of transposable elements (TEs), occurring through epigenetic reprogramming of the mammalian embryonic germline, requires the existence of an efficient mechanism coordinating the piwi/piRNA pathway and the *de novo* DNA methylation machinery. Accumulating evidence indicates that piRNA-binding, mammalian piwi homologues such as Miwi, Miwi2, and Mili proteins, are required for the germline maintenance and spermatogenesis in *Drosophila* and the mouse (Thomson & Lin, 2009). This view is supported by the fact that piRNAs are expressed in mouse male germ cells, particularly in pachytene spermatocytes and round spermatids (Klattenhoff & Theurkauf, 2008). Deficiency of Mili and Miwi2 leads to arrest of gametogenesis and complete sterility in males (Aravin et al., 2007; Carmell et al., 2007; Kuramochi-Miyagawa et al. 2008). Thus, it is likely that Mili and Miwi2 partners, the piRNAs, are potentially involved in regulating the meiotic and postmeiotic processes of male germ cell development, as they do in the fly. There are hundreds of thousands if not millions of individual piRNA genes which are transcribed from piRNA clusters from several, up to 200, kilobases in length. Each cluster contain multiple sequences that generate piRNAs. Moreover, in a number of clusters transcription occurs in both directions. It is currently unclear how primary piRNA are produced from piRNA clusters. They are not produced by Dicer enzymes and do not require formation of stable double-stranded RNA intermediates (Vagin et al., 2006). There are two alternative and distinct pathways of piRNA biogenesis. The first one is primary piRNA biogenesis and the second one is the so called "ping-pong amplification loop" pathway. These pathways are conserved in many animal species (Houwing et al., 2008; Kawaoka et al. 2009; Lau et al. 2006; Robine et al. 2009). In the mouse pro-spermatogonia, the primary piRNAs are produced through primary processing which generates an initial pool of piRNAs that target many TEs. Thereafter they are amplified through the ping-pong pathway, which requires slicer activity of Mili and Miwi2 proteins. In the ping-pong cycle, piRNA molecules that target active TE elements are highly amplified (for review see Siomi et al. 2011). In mouse spermatocytes, primary piRNA

biogenesis is the only process that generates piRNA, whereas in pre-meiotic spermatogonia, specific sequences generated by the primary pathway are amplified in the ping-pong cycle. In the mouse, Mili and Miwi2 function in the ping-pong cycle (Aravin et al., 2007). The piRISC complexes loaded by piRNA silence complementary RNA targets. Silencing occurs by cleavage and also through some chromatin effects (Nishida et al.; 2007; for review see Thomson & Lin, 2009). Deficiency of piwi proteins in flies, but also in mice, leads to the lack of primary piRNA, although it is unlikely that piwi-proteins are directly involved in primary piRNA processing. Finally, the piRNA pathway phenomenon opens a new fascinating field for exploration of potential roles of piRNAs in human germ cell development, infertility and the origin of TGCTs.

Therefore, to get more insight into the potential contribution of the piRNA pathway in human male infertility, the genetic variation of several piwi homologues, HIWI, HILI, HIWI3 and HIWI2, was studied in 490 patients with idiopathic azoospermia or oligozoospermia and in 468 fertile males from a Chinese Han population. Among 9 SNPs identified in the patients, 2 exhibited a significant association with the risk of oligozoospermia under a dominant model (variant containing versus homozygous wild-type genotype). Although the biological effects of one of these SNPs, a T->C transition (rs508485) in *HIWI2* 3'UTR was not clear, individuals carrying this transition in at least one allele, had a significantly increased risk of oligozoospermia. In contrast, individuals carrying a non-synonymous rs11703684 Val471IIe mutation in the *HIWI3* gene exhibited a borderline significantly reduced oligozoospermia risk compared with individuals with the wild-type genotype. After classification according the age or smoking status, a protective effect of the Val471IIe mutation on spermatogenesis was more obvious in non-smokers. Interestingly, the Val471IIe mutation was located within the PIWI functional domain which is crucial for RNA-mediated silencing in germ cells. While the function of HIWI3 protein in human spermatogenesis remains unclear, these finding may suggest a significant role of the piwi homologue in spermatogenesis. Altogether, the genetic variations identified in this study are more likely to be associated with oligozoospermia rather than azoospermia (Gu et al., 2010). Certainly, more studies are needed in various worldwide populations to properly assess the contribution of the piRNA pathway to defects in human male infertility.

6. Future directions

A tremendous technological advance in screening for male infertility causing mutations has been made in the recent years, enabling the collection of a large amount of data. Paradoxally, the latest advances in understanding posttranscriptional mechanisms of gene expression regulation in germ cells has revealed that a search for male infertility causing defects is even more complex than it had been expected several years ago. It is clear nowadays, that a gene can be disrupted not only by a mutation occurring within the open reading frame or promoter of a specific gene, but also within it's 3'UTR. This region seems to be rich in signals necessary for proper expression. First of all, it contains recognition signals for specific RNA-binding proteins. This seems particularly important in the light of a recent hypothesis that mRNAs that encode functionally related proteins are coordinately regulated during development and form the so-called posttranscriptional RNA regulons, involving specific complexes of RNA-binding proteins (Keene, 2007). One can imagine that point mutations targeting these specific nucleotide motifs in 3'UTR may disrupt protein

recognition causing a severe mRNA deregulation. This notion is further compounded by the presence in 3'UTRs of small regulatory RNA recognition sites, including those for miRNAs. As summarized in this chapter, there is a high probability that male infertility can be induced by point mutations within miRNA recognition sites. It has been recently demonstrated that a single nucleotide point mutation within the recognition site for a specific microRNA caused Crohn disease (Brest et al., 2010). Involvement of miRNAs in male infertility has been poorly assessed so far but may be quite complex. It is known that miRNAs can function in a combinatorial manner, given that an mRNA molecule may contain recognition sites for several miRNAs. On the other hand, each miRNA may repress up to hundreds of mRNAs. According to a recent, competing endogenous RNAs (ceRNA) hypothesis, mRNAs, transcribed pseudogenes, and long noncoding RNAs cooperate using miRNA recognition sites. These form large-scale regulatory networks across the transcriptome (Salmena et al., 2011). Dissection of these basic processes is certainly very important for a better understanding of germ cell development and male infertility.

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8. References

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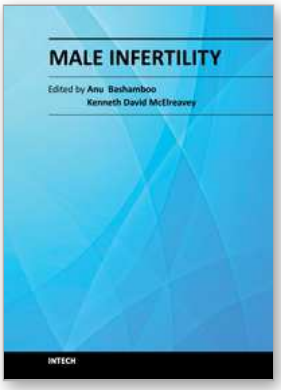

Male Infertility

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Male infertility is a multifaceted disease where genetic, epigenetic and environmental factors all contribute to the development of the phenotype. In recent years, there has been an increasing concern about a decline in reproductive health, paralleled by an increase in demand for infertility treatments. This calls for a detailed and thorough understanding of normal and aberrant testicular function and the environmental influences on the establishment and integrity of the male germ cell. This is crucial for understanding the complex pathophysiology of male infertility and eventual success of Assisted Reproductive Technologies.

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