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# Y chromosome and male infertility: Update, 2006

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## 1. ABSTRACT

Male factor infertility accounts for about half the cases of couple infertility and in around 50% of cases its etiology remains unknown. Molecular genetic techniques have unveiled a number of etiopathogenetic factors, including microdeletions of the Yq. Y chromosome microdeletions removing the AZoospermia Factor (AZF) regions are the most frequent molecular genetic causes of oligo/azoospermia. The intense effort of many laboratories contributed to a better understanding of the clinical significance of this genetic anomaly and to the identification of fertility candidate genes in the AZF regions. Important progress has been made on the structure of the Y chromosome and the mechanism of deletion. Studies aimed to define a predisposing genetic background for Yq deletions were not successful, perhaps due to the low number of patients analyzed so far. The screening for Yq deletions became a routine diagnostic test that provides an etiology for spermatogenic disturbances, and assess in the prognosis for testicular sperm retrieval according to the type of deletion. Assisted reproductive techniques represent an efficient symptomatic therapy for men bearing Y microdeletions, however, this genetic defect is transmitted to the male offsprings, affecting their fertility. Future studies should focus on understanding the biological function of AZF genes which is an essential step for the development of more appropriate and knowledge-based therapies.

## 2. INTRODUCTION

The first review on Y chromosome and male infertility appeared in the Frontiers in Bioscience in 1999 (1), 3 years after the molecular definition of the three AZF regions (2). At that time, there were already about 20 publications that described large scale screening for Yq deletions in infertile men. The clinical definition of patients and the markers used for the screening were heterogeneous and consequently, many important clinical questions were still unresolved. At that time, it was still unclear what the real frequency of the AZF deletions was and if there were any ethnic or geographic differences or any predisposing Y background. Concerns were raised about the existence of a genotype-phenotype correlation and about the specificity of AZF deletions in spermatogenic failure.

However, despite controversies, we pointed out at that time that the diagnosis of Yq deletions has an important clinical value since it provides the etiology of spermatogenic failure and enables one to avoid unnecessary medical or surgical treatments. Moreover, since a progressive decrease of sperm count over time was observed in these patients, we proposed sperm cryoconservation for both the patient and for his future son, as a preventive therapy. In the same year, the first EAA guidelines for Y chromosome deletion screening was also published which has clearly indicated the need for using a reliable set of markers for routine analysis (3). At the time

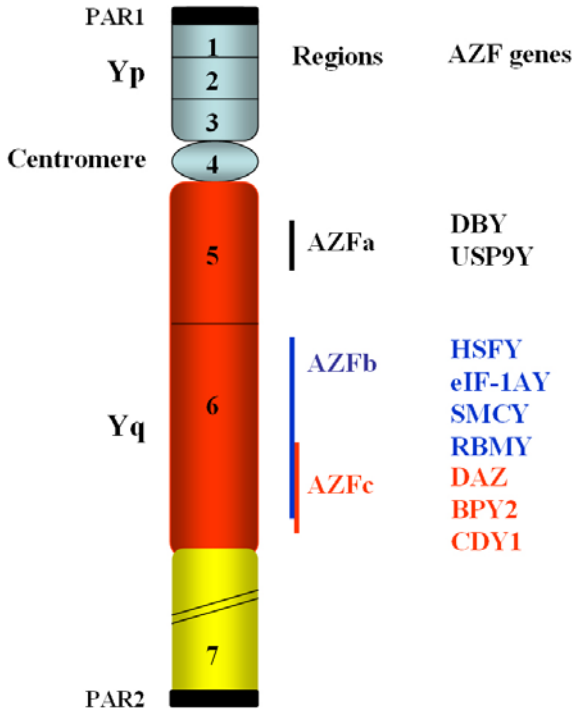


**Figure 1.** Schematic representation of the Y chromosome showing the 7 deletion intervals. The three AZF regions and the candidate AZF genes are indicated. PseudoAutosomial Region 1 and 2 (PAR1, PAR2); Yp: short arm of the Y chromosome; Yq: long arm of the Y.

that **the** majority of the AZF genes were identified (4), we were confident that insights into the function of the AZF genes would have been achieved very soon.

By the beginning of 2005, many of the above mentioned clinical questions were answered and thanks to the EAA guidelines and EAA/EMQN external quality control programme (5, 6) Yq testing has become more homogeneous and reliable in different laboratories. The complete Y sequence and gene /transcript content was published and the mechanism by which AZF deletions take place was fully understood (7). Recently, new deletion types with yet uncertain clinical meaning have also been reported opening a new area of research in this field (8, 9 and references therein).

Unfortunately, our knowledge concerning the biological function of the AZF genes has only slowly progressed during the last 6 years and the time for a knowledge-based aetiological therapy seems to be still very far away. An updated analysis of the available data on the Y chromosome and male infertility including both clinical and basic research is the subject of the present review.

## 3. Y CHROMOSOME STRUCTURE

The Y chromosome comprises only 2-3% of the haploid genome. It is an acrocentric chromosome and consequently contains a short arm (Yp) and long arm (Yq), demarcated by a centromeric region essential for chromosome segregation (Figure 1). The human Y chromosome is classically divided into two functionally distinct regions: i) the pseudoautosomal regions (PAR1 and PAR2), which are homologous with X chromosome sequences and are responsible for correct pairing between the two sex chromosomes during male meiosis; ii) the male specific region Y (MSY), previously called the "Non-recombining region Y" (NRY), in which, in certain parts, instead of classical recombination "intrachromosomal gene conversion" (non-reciprocal transfer) takes place (10). Thanks to the presence of this conversion–based system of gene copy "correction", a certain number of Y genes have been preserved from the gradual accumulation of deleterious mutations ensuring their continuity in time (7,10). This region comprises 95% of the length of the chromosome.

The extent of the MSY region is roughly 63Mb, but only 23Mb are transcriptionally active (euchromatic portion). The heterochromatic region comprises distal Yq. This region is polymorphic (variation in length) within males, constituting almost half the chromosome in some men while being undetectable in some others. This part of the chromosome is assumed to be genetically inert and it is mainly composed of two highly repetitive sequences families, DYZ1 and DYZ2, containing about 5000 and 2000 copies of each respectively.

The euchromatic region contains three classes of sequences, named by Page and collegues X-transposed, X-degenerate and ampliconic (7). The X transposed sequences are 99% identical to DNA sequences in Xq21 and derive from a massive X to Y transposition that occurred about 3-4 million years ago. These sequences contain the highest density of repeats (interspersed repeat elements) and only two genes with homologues in the Xp21. The X-degenerate segments are relatively rich of single copy gene or pseudogene homologues of X linked genes. The third class of sequences, ampliconic segments, are composed by long MSY specific repeat units named "amplicons" which show as much as 99,9% of intrachromosomal identity. These segments exhibit the highest density of genes, both coding and non-coding, among the three sequence classes.

The MSY euchromatin contains a total of 156 transcriptions units with 78 protein coding units and 78 putative non coding units (7). The protein coding units are the product of 27 genes, 12 of which are expressed ubiquitously and 11 are exclusively, or predominantly, expressed in testes. All 12 ubiquitously expressed genes of the MSY reside in the so called X-degenerate sequence whereas the majority of genes with testis specific expression are located in the ampliconic segments.

The majority of the MSY genes are involved in male specific functions, such as male sex determination (SRY) and spermatogenesis (i.e. genes of the AZoospermia Factor regions of the long arm of the Y chromosome). Consequently mutations/deletions of these genes lead to sex reversal or spermatogenic failure (1, 12). In addition, loss or rearrangements of the Y are also associated to a

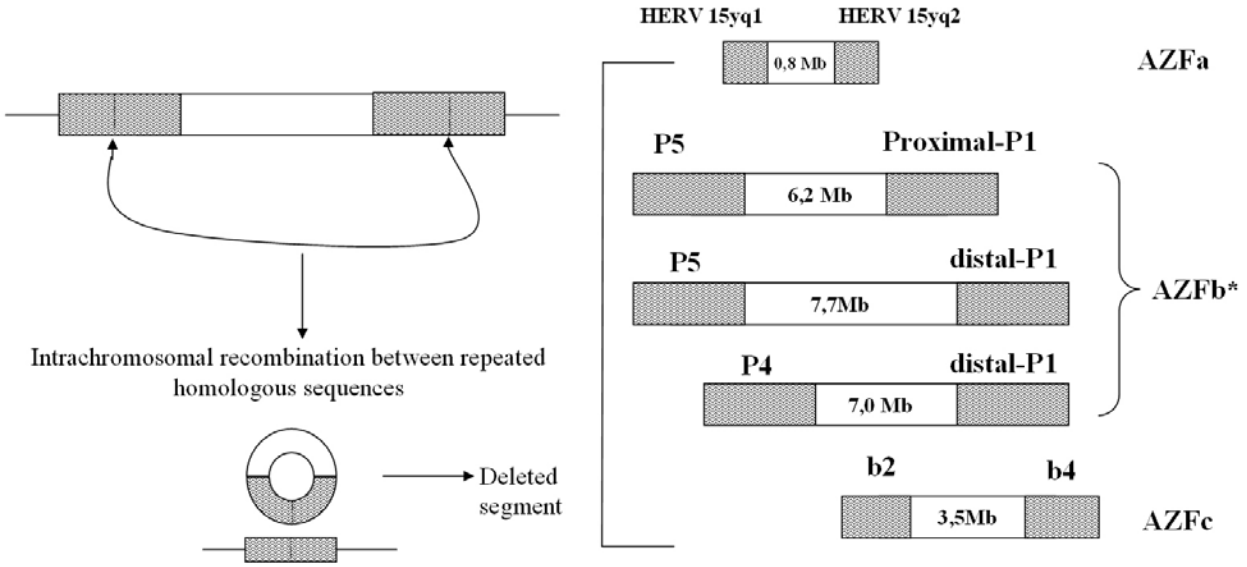


**Figure 2.** Schematic representation of the mechanism of AZF deletions. Boxes with motifs represent repeated homologous sequences situated at the border of recurrently deleted regions on the Yq. AZFa deletions occur between two homologous retroviral sequences (HERV15q). AZFb* deletions partially overlap with the AZFc region and may take place between different palindromic sequences (P5, P4, P1). The classical AZFc deletion occurs between two repeated sequences b2/b4 entirely situated in the AZFc region. The extent of the deleted segment is indicated in each type of AZF deletion (figure not in scale).

number of other phenotypes, such as Turner stigmata (13), skeletal anomalies (14) and the development of a rare type of gonadal cancer (gonadoblastoma) (15). The MSY also contains genes with housekeeping cellular activities (7,16) and are probably involved in functions other than male reproduction. The same genes may be responsible of the sexual dimorphism observed for certain pathologies such as hypertension, autism etc. (for review see 17 and references therein).

## 4. Yq DELETIONS AND SPERMATOGENESIS

The first association between azoospermia (absence of spermatozoa in the ejaculate) and microscopically detectable deletions of the long arm of the Y chromosome has been demonstrated by Tiepolo and Zuffardi in 1976 (18). Since in 4 patients the deletion was *de novo* (their fathers were tested and found to carry intact Y chromosome) they proposed the existence of a spermatogenesis factor, the AZoospermia factor (AZF) encoded by a gene on distal Yq. Successively, the development of molecular genetic tools, firstly Y specific DNA probes (19) than sequence tagged sites (STS: short tracts of DNA that acts as a landmark to define position on a physical map) (20), permitted to narrow the extension of the AZF region. Using the above mentioned molecular tools the breakpoints of a large panel of infertile men displaying a cytogenetically visible abnormality in Yq lead to the construction of molecular interval maps. Many STS based screening have been undertaken in patients affected by azoo/severe oligozoospermia in order to define the AZF locus and isolate candidate genes for AZF. Vogt et al. (1) observed that Yq microdeletions follow a certain deletion pattern, with three recurrently deleted nonoverlapping subregions in proximal, middle and distal Yq11, designated AZFa, AZFb and AZFc, respectively. Only several years after the discovery of the three AZF regions, with the precise knowledge of the Y structure in Yq11, is became evident that the AZFb and AZFc regions are overlapping (10). New deletion patterns in the AZFb/AZFc regions have been proposed, however since data in the literature are based on markers which are unable to distinguish between these subtypes, it is more convenient to refer to the three "classical" deletion intervals. (Figure 1)

## 5. GENES AND GENE FAMILIES OF THE AZF REGIONS

### 5.1. AZFa region

AZFa region is located in proximal Yq within deletion interval 5. In 1999, it was estimated that the region is between 1 and 3 Mb in size and contains four single copy genes, namely USP9Y (former DFFRY), DBY, UTY and TB4Y. After the discovery of the mechanism of the AZFa deletion formation (see below) it became evident that its size is of 0,8 Mb (Figure 2) and contains only two single copy genes: USP9Y and DBY (21,22). The USP9Y protein is an ubiquitinyl hydrolase and belongs to the family C19 (cystein peptidases) which are intracellular peptidases that remove ubiquitin molecules from polyubiquitinated peptides. It may therefore play an important role at the level of protein turnover by preventing degradation of proteins through the removal of conjugated ubiquitin.

The DBY gene is predicted to encode an RNA helicase and thus is involved in RNA metabolism. Both genes are transcribed in multiple tissues and have a homologue on the X chromosome which escapes X inactivation. Protein expression analysis for the DBY gene allowed the identification of a translational control

mechanism. Vogt and coworkers reported (23) that despite the ubiquitous transcription of the DBY gene, its mRNA is translated specifically in the testis tissue and in particular in spermatogonia and pachytene spermatocytes.

**5.2. AZFb region**

Similar to the AZFa region, the discovery of the deletion mechanisms in concert with the fine definition of the Yq structure led to a fundamental change of our view concerning the extension of the AZFb region. It became clear that the classical AZFb region is partially overlapping with the AZFc region. Previously its estimated size was around 1-3 Mb, now we know that there are two possible recombination sites and the extent may vary from 6,2 to 7,7Mb (10) (Figure 2).

It contains genes which are present only in the AZFb region (HSFY, eIF-1Y, SMCY) and others which are shared with the AZFc region (one copy of BPY2 and CDY, two copies of DAZ). Moreover, two gene families with multiple copies on the Y have their active copies in the AZFb region: RBMY and PRY. More than 30 RBM genes and pseudogenes are spread over both arms of the Y chromosome (24-26). The AZFb region contains at least one functional RBM copy, located in the distal portion of this deletion interval (27) RBMY (RNA Binding Motif Y) is a testis specific splicing factor and it is homologous to RBMX on the X chromosome (hnRNPG) and to a functional retrogene on chromosome 11 (11p15) hnRNPG-T. Both the X and the Y derived protein are highly expressed in mitotically active cells and thus may promote mitotic divisions in spermatogonia (28, for review see 29,30 and references therein). Beside spermatogonia, the RBMY protein is expressed also in the nuclei of spermatocytes and round spermatids.

Two functional copies of the PRY gene are present in the AZFb region and encodes a protein phosphatase which is probably involved in apoptosis of defective spermatozoa (31). The expression pattern of this protein (in spermatids and spermatozoa) indicates a role in the post-meiotic phases of spermatogenesis.

The HSFY (Heat Shock Factor Y) gene is situated in the palindromic sequence P4 in proximal AZFb and there are two copies. This gene encodes a novel heat shock protein which is specifically expressed in Sertoli and germ cells which exhibits a stage-dependent translocation from the cytoplasm to the nucleus in spermatogenic cells during spermatogenesis (32). The gene has a homologue HSP2 on 6p22 which codes for a heat shock transcription factor. The eIF-1AY encodes a Y isoform of a eIF-1A, an essential translation initiation factor. eIF-1AY has been mapped to interval 5q and subsequently defined between sY127-sY129 (33). This gene has an X homologue (eIF-1AX) which escapes inactivation. Both X and Y homologoues are ubiquitously expressed, with high level of expression in the testis (4). The biological function of SMCY is unknown but it encodes a male specific histocompatibility antigen (H-Y).

**5.3. AZFc region**

The region is 3,5 Mb and in analogy to the other two AZF regions it is delimited by two repeated sequences (b2 and b4) (Figure 2).

The first gene identified in the AZFc region is the DAZ gene (34). It is a multicopy gene with two copies situated in P2 (DAZ1/DAZ2) and two located 1.47 Mb distant in the large P1 palindrome (DAZ3/DAZ4). The DAZ gene has been transposed and pruned (amplified) from chromosome 3 (3p25; DAZL1 locus) after divergence of the Old world monkey line of primates (35, 36). The structure of the 4 copies is variable especially in the number of exon 7 variants which is also known as the "DAZ-repeat" (37, 38). The DAZ gene family includes two autosomal homologues, DAZL on chromosome 3 and BOULE on chromosome 2 (39). The finding that the human DAZ transgene is able to partially rescue the spermatogenic failure of mice homozygous for a null allele of the Dazl gene (40) suggests a possible interplay between DAZL and DAZ in humans.

All DAZ gene family members are encoding testis specific RNA binding proteins and thus are probably involved in the translational control of transcripts with spermatogenic function (for review see 41). Studies in flies demonstrate that the BOULE gene encodes a key factor of meiosis in male germ cells, regulating the expression of twine, a cdc25 phosphatase, which promotes progression through meiosis (42). In analogy, a translational control of CDC25 transcripts by BOULE has been reported also in human (43) and human BOULE transgene is able to rescue the meiotic block in boule mutant flies (44). DAZL and DAZ both interact with the DAZAP1 protein (Deleted in Azoospermia Associated Protein 1) which shuttles between the nucleus and the cytoplasm and may play a role in mRNA transport and/or localization (45). Expression analysis of the DAZ protein gave discordant results probably due to the use of different polyclonal DAZ-antisera. In one study, DAZ mRNA was detected in early spermatids whereas the protein was observed in late spermatids and spermatozoa. (46). In an other study DAZ expression has been found in multiple cell compartments at multiple points in male germ cell development (47). In this study, human DAZ and human and mouse DAZL proteins were present in both the nuclei and cytoplasm of foetal gonocytes and in spermatogonial nuclei. Interestingly, the proteins relocate to the cytoplasm during male meiosis. Human DAZL protein (but not DAZ) persists in spermatids and even spermatozoa. These results would suggest that DAZ proteins may act during meiosis and much earlier, when spermatogonial stem cell populations are established. The observed protein-protein interaction between DAZL and Pumilio-2 (PUM2) also suggest an important role in fetal germ cell development (48).

The CDY gene family has a similar origin like the DAZ gene i.e. it was retrotransposed from the CDYL locus on chromosome 6 (4). While the autosomal homologue is expressed ubiquitously, the CDY gene is specifically expressed in the testis. Four alternative spliced transcripts encoding three different protein isoforms have

been described (49). CDY are nuclear proteins observed in spermatids and show histone acetyl transferase activity with a strong preference for H4 (50). The Basic Protein Y 2 (BPY2) gene is expressed exclusively in the testis. The BPY2 protein represent singleton without detectable sequence homologues. The analysis of the 3D protein structure predicted a possible role of this highly charged protein in DNA or RNA binding (51).

A part from protein coding genes the AZF regions contain two gene families with unknown protein coding potential: i) the three members of the CYorf family (CYorf14, CYorf15A, CYorf15B) which are all expressed in multiple tissues and have homologues on the X (cXorf 14, cXorf15, cXorf15). ii) The TTY (Testis Specific Transcription Y) family which are considered transcription units with no significant open reading frame. Members of this family are present also outside of the AZF regions on both the short and the long arm of the Y.

For the moment it is not known whether all genes situated in the AZF regions are important for spermatogenesis. Since deletions occur in block- removing more than one gene- the role of a single AZF gene cannot be extrapolated from the deletion phenotype. To date only one confirmed gene specific deletion has been reported in the literature in which the breakpoint of the mutation has been defined (52). The associated phenotype i.e. hypospermatogenesis indicates that this gene, USP9Y, is probably not essential for the initiation and completion of spermatogenesis and it has probably a role in enhancing the quality and efficiency of spermatogenesis. Other authors have also reported single gene deletions (DBY, USP9Y, HSF-Y) however these studies did not define the deletion breakpoints (53,54). Since polymorphisms, rearrangements may lead to false deletions these data await further confirmation.

## 6. MECHANISM OF AZF DELETIONS

The mechanism by which classical AZF deletions take place is through intrachromosomal recombination between homologous sequences (7, 55). The long arm is indeed composed of numerous Y specific repetitive sequence blocks (called “amplicons”). These amplicons are assembled in eight palindrome structures (P1-P8) and are highly symmetrical. The homologous amplicons mapped in the different palindrome arms revealed extensive homologies between 99.940 and 99.997%. The AZFb and AZFc deletions occur between palindromes whereas the AZFa deletion is the result of intrachromosomal recombination events between two repetitive specific HERV15 (Human Endogenous Retroviral #15) sequence blocks (22, 23). (Figure 2)

## 7. AZF DELETIONS AND Y CHROMOSOME BACKGROUND

Polymorphic markers on the MSY region define Y chromosome lineages (or haplogroups) which are monophyletic groups of Y chromosomes sharing allelic states at slowly mutating binary markers. Until now, more than 200 Y specific single nucleotide polymorphisms (SNPs) has been identified providing, together with a number of indels (insertions/deletions), a detailed and robust phylogenetic tree of Y chromosomal lineages (56,57). The absence of recombination on the MSY region makes that polymorphisms located in this region are in tight association with potential functional variations associated with Y-linked phenotypes.

A predisposing Y background to AZF deletions has been hypothesized on the basis of a previous study reporting a Y haplogroup that is susceptible to aberrant X/Y exchange during male meiosis, leading to the XX male syndrome (58). Since the mechanism by which AZF microdeletions originate can be traced to intrachromosomal recombination between repetitive elements (22, 23, 55), sequence differences or different orientation of the repeated blocks may predispose or protect against specific type of deletions. An example is the polymorphic deletion of L1PA4 element which confers a higher homology between the two retroviral blocks in the flanking sequences of the AZFa region and thus may, in theory, facilitate homologous recombination. This deletion is considered as a polymorphism and reach high frequencies in populations from the Middle East. Similarly to AZFa region, there could be differing susceptibilities to the other AZF deletions depending on the Y chromosome structure. Men carrying Y chromosome microdeletions from Ireland, Scotland, Germany, Italy, Spain, Holland and Denmark were haplotyped and the haplotype distribution was then compared to infertile men without microdeletions (59) or compared to an unselected male control population (60). Y chromosome microdeletions were found to occur on different Y chromosome backgrounds and there was no significant difference between Y chromosome haplogroup distribution in the study and control groups. However, due to the relative rarity of AZFa deletions (5% of the total deletions), the number of this type of deletions included in both studies was small and, despite the apparent lack of association, the relationship between Y-chromosome background and microdeletion formation cannot be ruled out.

Different Y chromosome backgrounds may also be responsible for the inter-individual variation in the phenotypic expression of a given AZF deletion such as the complete AZFc deletion which can be associated with both severe oligospermia or with the total absence of spermatogenic cells in the testis. Future clinical-molecular combined studies are needed to accredit this attractive hypothesis.

A part from AZF deletions other Y related factors such as partial gene copy deletions of multicopy genes or rearrangements/inversions occurring in non coding sequences but with possible functional effects on gene expression may influence spermatogenesis. These type of alterations may segregate with certain Y backgrounds, therefore a role for Y chromosome background in determining spermatogenic potential has also been proposed. This hypothesis was confirmed in the Danish and Japanese population whereas not in others (for review see 17 and references therein).

## 8. CLINICAL ASPECTS OF Yq DELETIONS

The clinical significance of Yq deletions have been debated for long time mainly because of the large variability in deletion frequencies reported by different authors and because Yq deletions have also been reported in "fertile" men. Deletions which are clinically relevant remove partially or most frequently completely one or more AZF regions and they represent the most frequent molecular genetic cause of oligo/azoospermia.

### 8.1. Y deletions are specific for spermatogenic disturbances

In 12 men out of 2295 fertile controls, small deletions outside the DAZ region have been reported (for review see 61 and references therein). However, sperm analysis was not available in any of these cases making impossible to ascertain if these men had also normal spermatogenesis. About 10% of "fertile" men are severe oligospermic (62), therefore fertility is not a synonym of normozoospermia. Despite this concept, most studies used "proven fertile men" with unknown sperm count, as a control group rather than a group of "normospermic men". Thus the fertile men with deletions may themselves have been oligozoospermic or these deletions may be rare polymorphic variants (63,64) or related to technical errors. Natural transmission of deletions removing the entire AZFc deletions has been also reported (1, 65, 66, 67), but sperm analysis was available only in two cases. Two fathers (one with reduced sperm count) were able to father only one child (1, 65) whereas the father of four infertile sons was azoospermic many years later after the natural conception of his sons (66). These findings indicate that the natural transmission of this genetic defect is likely to be related to a situation where the father had oligozoospermia with progressive decrease of his sperm count over time associated with a high fertility state of the female partner.

To date over 1000 normospermic men have been tested in a double blind study (68) and in subsequent studies (see 61 and references therein, personal data) and no Yq microdeletions have been reported for this category of men. It is therefore clear that AZF deletions inevitably lead to spermatogenic disturbances of different entity.

### 8.2. Genotype/phenotype correlations

Since AZF genes are mainly expressed in the testis, a number of studies have been undertaken in order to clarify if AZF deletions may cause other testis related pathologies than spermatogenic failure. No final evidence for a cause–effect relationship with varicocele, cryptorchidism, testis cancer have been reported, on the contrary, a meta-analysis by Kunej et al. (69) showed that the incidence of Yq deletions in cryptorchid men is less frequent than in patients with normally descended testis. In a group of 437 patients we found a total of 15 AZF deletions, 80% of these patients were previously defined as idiopathic infertile whereas in a minority of cases other abnormal andrological findings such as varicocele, cryptorchidism, central hypogonadism, testis trauma etc were also present. In order to define if hormone levels may be indicative for the presence of Yq deletions, we and others performed a complete hormonal analysis in all patients screened for Yq deletions. In two studies of the Danish population (68, 70) FSH levels in patients with AZF deletions were above the mean value in all cases. Serum Inhibin B concentrations were uniformly below the normal range in each patient. In an other study, testicular endocrine function, in a total of 89 oligo-azoospermic patients with and without microdeletions, have been evaluated (Tomasi personal communication) and Inhibin B and FSH levels were undistinguishable in patients with idiopathic and microdeletion-associated oligo-azoospermia. These data do not support the hypothesis proposed by an other group (71), that microdeleted patients have a less severe impairment of Sertoli cell function than patients with idiopathic oligo-azoospermia. The relatively high level of Inhibin B, found in the group of men with AZF deletions presenting SCOS may be related to overlooking of areas of spermatocytic arrest or to a false overrepresentation of SCOS detected by fine needle biopsy used by authors (71).

In conclusion, a part from spermatogenic disturbance, no specific hormonal alterations or specific pathologies have been identified in association with Yq deletetions. Since the first description of the three AZF regions Vogt et al. (1) suggested that in each subregion the deletion is associated with distinct histopathological profile and that azoospermia is a constant phenotype in men with AZFa and AZFb deletions. This initial genotype-phenotype correlation has been questioned by subsequent studies reporting the presence of spermatozoa in the ejaculate or in the testis in patients with AZFa and AZFb deletions. These apparent discrepancies have mainly derived from the incorrect use of terminology. A deletion *in* the AZFa or AZFb region or a deletion *of* the AZFa or AZFb region are two different conditions and these two terms were often used as interchangeable. The first terminology should be used in case the deletion does not remove the entire region whereas the second only in case of a *complete* deletion (when it is of the same size described by Vogt et al. 1). Deletions removing the entire AZFa or AZFb regions ("complete" deletions) are associated with Sertoli Cell Only Syndrome (SCOS) and spermatogenic arrest, respectively (72,73). On the contrary, such a strict genotype/phenotype correlation is lacking for the partial deletions of these regions or for the AZFc deletions which are associated with a variable phenotype ranging from hypospermatogenesis (oligozoospermia) to SCOS (azoospermia). A possible explanation for such a variable phenotype is a progressive regression of the germinal epithelium (74, 75) or a progressive decrease of sperm number over time (76, 77, 78). An alternative explanation for the variable phenotype can be related to influences of the genetic background (compensation for the absence of Yq genes, by autosomal or X-linked factors), the presence of 45X lines (79) and environmental factors in different individuals.

### 8. 3. Incidence of AZF and AZF gene specific deletions. Indications for screening.

The initial large variability of deletion frequencies reported in the previous review in 1999 was more likely the consequence of technical problems and the

use of unreliable markers rather than an expression of true ethnic differences. With time, after having improved the detection method this figure became more homogeneous. Although the composition of the study population is certainly the major factor influencing deletion frequency, the originally reported 55% of deletion frequency in patients affected by SCOS was clearly an overestimation and was not confirmed by later studies (6).We studied 4 different populations (three of European and one of non-European origin) with a similar set of primers and using homogeneous criteria for the definition of "idiopathic" and "non-idiopathic" infertility (68, 80, 81 and personal data). The deletion frequency calculated for the overall study population of each study ranged from 1.5% to 10.8%. The highest frequencies were found in two studies with the largest number of azoospermic men included. The calculation of deletion frequency for uniformly defined group of idiopathic severe oligo- and azoospermic men showed a more homogeneous figure, of 10-18% which suggest that ethnic or geographic differences apparently have no influence on deletion frequency. The highest incidence, 15% was found in idiopathic azoospermic men whereas in the group of severe oligospermic patients it drops to 5-7%.

Despite the effort of many laboratories, isolated AZF gene deletions have been reported only sporadically therefore they must be extremely rare. Interestingly, a group (53) have reported a surprisingly high number of isolated gene-specific deletions within the AZFa region in a relatively small series of patients. This finding has not been confirmed in more than 1300 infertile males tested for AZFa genes by different groups (52, 68, 70, 80, 81). Considering that no specific phenotype has been reported for patients bearing "isolated gene" deletions, it is unlikely that the lack of isolated gene deletions in the other studies is related to patient selection criteria. In our own cohort of patients from the Florence area we tested a total 800 men for the following AZF genes: USP9Y and DBY (AZFa); HSFY and eIF-1AY (AZFb). So far we found one patient with a partial deletion of the USP9Y gene (82 submitted). Recently a deletion removing one AZFb gene, the HSFY (54), has been reported in an azoospermic man, but in analogy to the majority of isolated AZFa gene deletions, the molecular definition of the deletion is incomplete and rearrangements influencing PCR based detection has not been ruled out.

As far as the AZF deletions are concerned, it can be now concluded that: i)Y deletions have been found almost exclusively in patients with <1 millions spermatozoa/ml; ii) deletions are extremely rare with a sperm concentration >5 millions of spermatozoa/ml (approximately 0.7%) and in certain cases deletions removed only single STSs which without further confirmation by other techniques, such as Southern blotting or breakpoint definition, are of dubious significance; iii) the most frequently deleted region is AZFc (approximately 60%), followed by deletions of the AZFb and AZFb+c or AZFa+b+c regions (35 %) whereas deletions of the AZFa region are extremely rare (5%). Indications for screening AZF deletions are azoospermia and severe oligozoospermia (<5 million spermatozoa/ml). Since isolated gene specific deletions are extremely rare there is no absolute requirement for gene specific deletion screening. The EAA guidelines provides a set of primers (two markers for each region) which is able to detect over 95% of clinically relevant deletions (6).

**8. 4. Yq deletions and genetic counselling.**

A part from a few inherited cases, the majority of Yq deletions occur as *de novo* events. While the mechanism of Y chromosome deletions is now understood, their cellular origin is still unclear. A testicular origin (during meiosis) seems the most likely although deletions could arise in fertilised eggs or embryos. The natural transmission of AZFc and partial AZFb deletions indicates that the absence of gene products removed by these types of deletions does not preclude sperm fertilizing ability. For the moment the only effective treatment for these patients is IVF (in vitro fertilization with embryo transfer) or ICSI (IntraCytoplasmic Sperm Injection) depending on the quantity of ejaculated spermatozoa. Due to discordances in the literature, it is not clear if the fertilization rate and embryo development are comparable to that observed in men without deletions (83, 84, 85, 86, 87).

The screening for Yq deletions is of additional clinical utility in azoospermic men in which the type of deletion may have prognostic value for testicular sperm retrieval. Testicular sperm extraction (TESE) is not recommended in case of complete AZFa or AZFb deletions since the probability of the presence of mature sperm is virtually zero. Although the question about a progressive decrease of sperm production leading to azoospermia is debated (for review see 88 and references therein, 89) cryoconservation of sperm can be advised as a preventive therapy since it is a non-invasive procedure.

After conception, Y deletion is obligatory transmitted to the male offspring therefore genetic counselling is mandatory. The phenotype of son may vary substantially and the extent of spermatogenic failure cannot be predicted entirely due to different genetic background and the presence or absence of environmental factors with potential toxicity to reproductive function.

We and others have reported that a significant proportion of spermatozoa from men with Y microdeletion are nullisomic for sex chromosomes (79, 90). This result indicates a potential risk for the offspring to develop 45,X0 Turner's syndrome and other phenotypic anomalies associated with sex chromosome mosaicism, including ambiguous genitalia. The screening for Y chromosome microdeletions in patients bearing a mosaic 46XY/45X0 karyotype with sexual ambiguity and/or Turner stigmata has shown a relatively high incidence of AZFc deletions (33%; 91). Additional data (79, 92, 93) support that Yq microdeletions could be associated with an overall Y chromosomal instability leading to the formation of 45,X0 cell lines.

Despite this theoretical risk, the 36 babies (18 male and 18 female) born from fathers affected by Yq

microdeletions are phenotypically normal (for references see 88). This could be due to the reduced implantation rate and a likely higher risk of spontaneous abortions of embryos bearing a 45,X0 karyotype.

In order to avoid the transfer of embryos with sex chromosome mosaicism, preimplantation diagnosis could be offered to the couple. This analysis, together with the abortion rate would provide a more realistic estimation about the real risks of 46, XY/45,X0 mosaicism and Turner syndrome.

## 9. CONCLUSIONS AND PERSPECTIVES

As we already suggested in 1999, the identification of Y deletions has a diagnostic, prognostic and preventive value. The diagnosis provides the cause of the spermatogenic failure and thus it avoids unnecessary medical (hormonal and non hormonal) and surgical (varicocele operations) treatments. In azoospermic men, the presence of a complete AZFa or AZFb deletion has a negative prognostic value for testicular sperm retrieval (73, 94, 95 and Silber personal comunication). In patients presenting oligozoospermia who are at risk for a progressive decrease of sperm concentration over time, cryoconservation of spermatozoa could avoid future more invasive techniques such as TESE/ICSI. The same preventive therapy may be proposed to affected sons (2).

A part from the classical AZF deletions, a new type of Yq deletion has recently attracted the attention of geneticist and andrologist. A partial deletion in the AZFc region, termed “gr/gr” has been described specifically in infertile men with varying degrees of spermatogenic failure (8, 96). This deletion removes half the AZFc gene content including two copies of the major AZFc candidate gene called DAZ (34). As it happens in the late 90thies for the classical AZF deletions, we are now facing to an intensive search for gr/gr deletions in infertile and control normospermic men in order to define their frequency and clinical significance (97, 98, 99, 100, 101). From the first studies it is already clear that in contrast to the classical AZF deletions, “gr/gr” deletions can be found also in normospermic men (98,100,101) although at a significantly lower frequency. Therefore, rather than a specific cause, this genetic anomaly represents a risk factor for spermatogenic failure. Among a number of plausible explanations for the heterogeneous phenotype we hypothesized the presence of polymorphisms or mutations in the autosomal homologue of the DAZ gene, DAZL (41). However, similarly to the AZFc deleted patients, we found no new mutations in the entire coding region of the DAZL gene and the polymorphic Thr12-Ala change (T12A), due to its relatively high frequency in the normospermic group, does not seem to have any modulating effect (101, 102). The currently used method for the detection of gr/gr deletions is based on STS plus/minus type of analysis which alone does not provide information about the type of missing gene copies. This analysis may also detect false deletions due to rearrangements of the STS containing sequence and is also unable to rule out a duplication of the non-deleted part of the AZFc region. The majority of “gr/gr” studies are lacking of a detailed molecular analysis i.e. the reduced gene dosage is not confirmed and the type of deleted gene copies is also unknown (98, 99, 99, 100). Mitchell and coworkers (97) have developed a method able to detect the type of the missing CDY and DAZ copies whereas Vogt and co-workers (103) developed an RFLP based DAZ copy analysis. Our preliminary data indicate that gr/gr deletions associated with the loss of CDY1a copy is found only in infertile men and not in normospermic control (101). However, the number of controls with gr/gr deletion is low and further combined studies are needed to confirm this preliminary result.

Besides the gr/gr deletions the AZFc region predisposes to a number of other possible *partial* deletions (9, 104). The Y chromosome background seems to play an important role in the pathogenic consequence of these deletions: for example another deletion named “b2/b3” (106) or “u3-gr/gr” (97) or “g1/g3” (107), which removes a similar quantity of AZFc genes than the gr/gr deletion, seems to have no effect on fertility status in association with a certain Y chromosome background commonly present in Northern Eurasian populations (Y haplogroup N) (106,107). A similar conclusion can also be drawn for the “gr/gr” deletion found in association with Hgr D2b which is present in 20% of Japanese men. Consequently, a combined molecular characterization (haplogroup, gene dosage, and gene copy type definition) of the “gr/gr” deleted patients and controls will probably allow the distinction between pathogenic and neutral deletions. In the meantime, the screening for “gr/gr” deletions maybe advised to patients attending for assisted reproductive techniques, since this test will provide the identification of a transmissible genetic risk factor for reduced sperm count.

In contrast to the advancement in the clinical aspects of AZF deletions, many fundamental questions concerning the function of AZF genes remains to be answered. The largely unsuccessful search for gene specific deletions/mutations did not allow to obtain information through genotype-phenotype correlation therefore future expression studies are needed to get insight into gene function. Partial AZFc deletions are promising new area of research which may lead to the identification of a new genetic cause (and not “only” a risk factor) of spermatogenic disturbance.

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## 11. REFERENCES


1. Krausz C & K. McElreavey: Y chromosome and male infertility. *Front Biosci.* 15,1–8 (1999)

2. Vogt PH, A. Edelmann, S. Kirsch, O. Henegariu, P. Hirschmann, F. Kiesewetter, F.M. Kohn, W.B. Schill, S. Farah, C. Ramos, M. Hartmann, W. Hartschuh, D. Meschede, H.M. Behre, A. Castel, E. Nieschlag, W. Weidner, H.J. Grone, A. Jung, W. Engel & G. Haidl: Human Y chromosome azoospermia factors (AZF) mapped to different subregions in Yq11. *Hum Mol Genet.* 5, 933-943 (1996)

3. Simoni M, E. Bakker, M.C. Eurlings, G. Matthijs, E. Moro, C.R. Muller & P.H Vogt: Laboratory guidelines for molecular diagnosis of Y-chromosomal microdeletions. *Int J Androl.* 22,292-299 (1999)

4. Lahn BT & D.C. Page : Functional coherence of the human Y chromosome*Science.* 278, 675-680 (1997)

5. Simoni M: Molecular diagnosis of Y chromosome microdeletions in Europe: state-of-the-art and quality control. *Hum Reprod.* 16, 402-409 (2001)

6. Simoni M, E. Bakker & C. Krausz: EAA/EMQN best practice guidelines for molecular diagnosis of y-chromosomal microdeletions. State of the art. *Int J Androl.* 27, 240-249 (2004)

7. Skaletsky H, T. Kuroda-Kawaguchi, P.J. Minx, H.S. Cordum, L. Hillier, L.G. Brown, S. Repping, T. Pyntikova, J. Ali, T. Bieri, A. Chinwalla, A. Delehaunty, K. Delehaunty, H. Du, G. Fewell, L. Fulton, R. Fulton, T. Graves, S.F. Hou, P. Latrielle, S. Leonard, E. Mardis, R. Maupin, J. McPherson, T. Miner, W. Nash, C. Nguyen, P. Ozersky, K. Pepin, S. Rock, T. Rohlfing, K. Scott, B. Schultz, C. Strong, A. Tin-Wollam, S.P. Yang, R.H. Waterston, R.K. Wilson, S. Rozen & D.C. Page: The male-specific region of the human Y chromosome is a mosaic of discrete sequence classes. *Nature*. 423,825-837 (2003)

8. Repping S, H. Skaletsky, L. Brown, S.K. Van Daalen, C.M. Korver, T. Pyntikova, T. Kuroda-Kawaguchi, J.W. De Vries, R.D. Oates, S. Silber, F. Van der Veen, D.C. Page & S. Rozen: Polymorphism for a 1.6-Mb deletion of the human Y chromosome persists through balance between recurrent mutation and haploid selection. *Nat Genet.* 35, 247-251 (2003)

9. Vogt PH: Genomic heterogeneity and instability of the AZF locus on the human Y chromosome. *Mol Cell Endocrinol.* 224, 1-9 (2004)

10. Repping S, H. Skaletsky, J. Lange, S. Silber, F. Van Der Veen, R.D. Oates, D.C. Page & S. Rozen: Recombination between palindromes P5 and P1 on the human Y chromosome causes massive deletions and spermatogenic failure. *Am J Hum Genet*. 71, 906-922 (2002)

11. Harley VR, M.J. Clarkson & A. Argentaro: The molecular action and regulation of the testis-determining factors, SRY (sex-determining region on the Y chromosome) and SOX9 [SRY-related high-mobility group (HMG) box 9]. *Endocr Rev*. 24, 466-487 (2003)

12. McElreavey K & C. Krausz: Sex Chromosome Genetics '99. Male infertility and the Y chromosome. *Am J Hum Genet*. 64, 928-933 (1999)

13. Zinn AR & J.L. Ross: Turner syndrome and haploinsufficiency. *Curr Opin Genet Dev*. 8, 322-327 (1998)

14. Rao E, B. Weiss, M. Fukami, A. Rump, B. Niesler, A. Mertz, K. Muroya, G. Binder, S. Kirsch, M. Winkelmann, G. Nordsiek, U. Heinrich, MH. Breuning, MB. Ranke, A. Rosenthal, T. Ogata, GA. Rappold. Pseudoautosomal deletions encompassing a novel homeobox gene cause growth failure in idiopathic short stature and Turner syndrome. *Nat Genet* 16, 54-63 (1997)

15. Lau YF: Gonadoblastoma, testicular and prostate cancers, and the TSPY gene. *Am J Hum Genet*. 64, 921-927 (1999)

16. Jobling MA & C. Tyler-Smith: The human Y chromosome: an evolutionary marker comes of age. *Nat Rev Genet* 4, 598-612 (2003)

17. Krausz C, L. Quintana-Murci & G. Forti: Y chromosome polymorphisms in medicine. *Ann Med.* 36, 573-583 (2004)

18. Tiepolo L & O. Zuffardi. Localization of factors controlling spermatogenesis in the nonfluorescent portion of the human Y chromosome long arm. *Hum. Genet*. 34, 119-124 (1976)

19. Vergnaud G, D.C. Page, M.C. Simmler, L. Brown, F. Rouyer, B. Noel, D.Botstein, A. de la Chapelle & J.Weissenbach J. A: deletion map of the human Y chromosome based on DNA hybridization. *Am J Hum Genet.* 38, 109-124 (1986)

20 Vollrath D, S. Foote, A. Hilton, L.G. Brown, P. Beer-Romero, J.S. Bogan & D.C. Page: The human Y chromosome: a 43-interval map based on naturally occurring deletions. *Science.* 258, 52-59 (1992)

21. Sun C, H. Skaletsky, S. Rozen, J. Gromoll, E. Nieschlag, R. Oates & D.C. Page: Deletion of azoospermia factor a (AZFa) region of human Y chromosome caused by recombination between HERV15 proviruses. *Hum Mol Genet*. 9, 2291-2296 (2000)

22. Kamp C, P. Hirschmann, H. Voss, K. Huellen & P.H. Vogt: Two long homologous retroviral sequence blocks in proximal Yq11 cause AZFa microdeletions as a result of intrachromosomal recombination events. *Hum Mol Genet*. 9, 2563-2572 (2000)

23. Ditton HJ, J. Zimmer, C. Kamp, E. Rajpert-De Meyts & P.H. Vogt: The AZFa gene DBY (DDX3Y) is widely transcribed but the protein is limited to the male germ cells by translation control. *Hum Mol Genet* 13, 2333-2341 (2004)

24. Ma K, J.D. Inglis, A. Sharkey, W.A. Bickmore, R.E. Hill, E.J. Prosser, R.M. Speed, E.J. Thomson, M. Jobling, K. Taylor, A: Y chromosome gene family with RNA-binding protein homology: candidates for the azoospermia factor AZF controlling human spermatogenesis. *Cell.* 75, 1287-1295 (1993)

25. Prosser J, J.D. Inglis, A. Condie, K .Ma, S. Kerr, R. Thakrar, K. Taylor, J.M Cameron & H.J. Cooke: Degeneracy in human multicopy RBM (YRRM), a candidate spermatogenesis gene. *Mamm Genome*. 7, 835-842 (1996)

26. Chai NN, E.C. Salido & P.H. Yen: Multiple functional copies of the RBM gene family, a spermatogenesis candidate on the human Y chromosome. *Genomics.* 45, 355-361 (1997)

27. Elliott DJ, M.R. Millar, K. Oghene, A. Ross, F. Kiesewetter, J. Pryor, M. McIntyre, T.B. Hargreave, P.T. Saunders, P.H. Vogt, A.C. Chandley & H. Cooke: Expression of RBM in the nuclei of human germ cells is dependent on a critical region of the Y chromosome long arm. *Proc Natl Acad Sci U S A*. 94, 3848-3853 (1997)

28 Elliott DJ, C.F. Bourgeois, A. Klink, J. Stevenin & H.J. Cooke: A mammalian germ cell-specific RNA-binding protein interacts with ubiquitously expressed proteins involved in splice site selection. *Proc Natl Acad Sci U S A*. 97, 5717-5122 (2000)

29 Elliott DJ: The role of potential splicing factors including RBMY, RBMX, hnRNPG-T and STAR proteins in spermatogenesis. *Int J Androl.* 27, 328-334 (2004)

30. Ehrmann I & D.J. Elliott: Post-transcriptional control in the male germ line. *Reprod Biomed Online.* 10, 55-63 (2005)

31 Stouffs K, W. Lissens, G. Verheyen, L. Van Landuyt, A. Goossens, H. Tournaye, A. Van Steirteghem & I. Liebaers: Expression pattern of the Y-linked PRY gene suggests a function in apoptosis but not in spermatogenesis. *Mol Hum Reprod.* 10, 15-21 (2004)

32 Shinka T, Y. Sato, G. Chen, T. Naroda, K. Kinoshita, Y. Unemi, K. Tsuji, K. Toida, T. Iwamoto & Y. Nakahori: Molecular characterization of heat shock-like factor encoded on the human Y chromosome, and implications for male infertility. *Biol Reprod.* 71, 297-306 (2004)

33. Krausz C, K. McElreavey, G. Weissenbach, G. Forti & C. Bishop: Identification and mapping of a novel Y chromosome isoform of eIF-1A. Miniposter book, 10th European Workshop on Molecular and cellular Endocrinology of the testis Capri 28/03-01-04 (1998)

34. Reijo R, T.Y. Lee, P. Salo, R. Alagappan, L.G. Brown, M. Rosenberg, S. Rozen, T. Jaffe, D. Straus, O. Hovatta, A. de la Chapelle, S. Silber & D.C. Page. Diverse spermatogenic defects in humans caused by Y chromosome deletions encompassing a novel RNA-binding protein gene. *Nat Genet.* 10, 383-393 (1995)

35. Shan Z, P. Hirschmann, T. Seebacher, A. Edelmann, A. Jauch, J. Morell, P. Urbitsch & P.H. Vogt: A SPGY copy homologous to the mouse gene Dazla and the Drosophila gene boule is autosomal and expressed only in the human male gonad. *Hum Mol Genet.* 5, 2005-2011 (1996)

36 Seboun E, S. Barbaux, T. Bourgeron, S. Nishi, A. Agulnik, M. Egashira, N. Nikkawa, C. Bishop, M. Fellous, K. McElreavey & M. Kasahara: Gene sequence, localization, and evolutionary conservation of DAZLA, candidate male sterility gene. *Genomics.* 41, 227-235 (1997)

37 Saxena R, L.G. Brown, T. Hawkins, R.K. Alagappan, H. Skaletsky, M.P. Reeve, R. Reijo, S. Rozen, M.B. Dinulos, C.M. Disteche & Page: The DAZ gene cluster on the human Y chromosome arose from an autosomal gene that was transposed, repeatedly amplified and pruned. *Nat Genet.* 14, 292-299 (1996)

38 Fernandes S, K. Huellen, J. Goncalves, H. Dukal, J. Zeisler, E. Rajpert De Meyts, N.E. Skakkebaek, B. Habermann, W. Krause, M. Sousa, A. Barros & P.H. Vogt: High frequency of DAZ1/DAZ2 gene deletions in patients with severe oligozoospermia. *Mol Hum Reprod.* 8, 286-298 (2002)

39. Xu EY, F.L. Moore & R.A. Pera: A gene family required for human germ cell development evolved from an ancient meiotic gene conserved in metazoans. *Proc Natl Acad Sci U S A.* 98,7414-7419 (2001)

40. Slee R, B. Grimes, R.M. Speed, M. Taggart, S.M. Maguire, A. Ross, N.I. McGill, P.T. Saunders & H.J. Cooke: A human DAZ transgene confers partial rescue of the mouse Dazl null phenotype. *Proc Natl Acad Sci U S A*. 96, 8040-8045 (1999)

41. Yen PH: Putative biological functions of the DAZ family. *Int J Androl.* 27,125-129 (2004)

42. Eberhart CG, J.Z. Maines & S.A. Wasserman: Meiotic cell cycle requirement for a fly homologue of human Deleted in Azoospermia. *Nature* 381, 783-785 (1996)

43. Luetjens CM, E.Y. Xu, R.A. Rejo Pera, A. Kamischke, E. Nieschlag & J. Gromoll: Association of meiotic arrest with lack of BOULE protein expression in infertile men. *J Clin Endocrinol Metab.* 89, 1926-1933 (2004)

44. Xu EY, D.F. Lee, A. Klebes, P.J. Turek, T.B. Kornberg & R.A. Reijo Pera: Human BOULE gene rescues meiotic defects in infertile flies. *Hum Mol Genet.* 12, 169-175 (2003)

45. Vera Y, T. Dai, A.P. Hikim, Y. Lue, E.C. Salido, R.S. Swerdloff & P.H. Yen: Deleted in azoospermia associated protein 1 shuttles between nucleus and cytoplasm during normal germ cell maturation. *J Androl.* 23,622-628 (2002)

46. Reijo RA, D.M. Dorfman, R. Slee, A.A. Renshaw, K.R. Loughlin, H. Cooke & D.C. Page: DAZ family proteins exist throughout male germ cell development and transit from nucleus to cytoplasm at meiosis in humans and mice. *Biol Reprod.* 63,1490-1496 (2000)

47. Habermann B, H.F. Mi, A. Edelmann, C. Bohring, I.T. Backert, F. Kiesewetter, G. Aumuller & P.H. Vogt: DAZ (Deleted in AZoospermia) genes encode proteins located in human late spermatids and in sperm tails. *Hum Reprod.* 13, 363-369 (1998)

48. Moore FL, J. Jaruzelska, M.S. Fox, J. Urano, M.T. Firpo, P.J. Turek, D.M. Dorfman & R.A. Pera: Human Pumilio-2 is expressed in embryonic stem cells and germ cells and interacts with DAZ (Deleted in AZoospermia) and DAZ-like proteins. *Proc Natl Acad Sci U S A.* 100, 538-43 (2003)

49. Kleiman SE, L. Yogev, R. Hauser, A. Botchan, B. Bar-Shira Maymon, L. Schreiber, G. Paz & H. Yavetz. Members of the CDY family have different expression patterns: CDY1 transcript have the best correlation with complete spermatogenesis. *Hum Genet.* 113, 486-492 (2003)

50. Lahn BT, Z.L. Tang, J. Zhou, R.J. Barndt, M. Parvine, C.D. Allis & D.C. Page: Previously uncharacterized histone acetyltransferases implicated in mammalian spermatogenesis. *Proc Natl Acad Sci U S A.* 99, 8707-8711 (2002)

51. Ginalski K, L. Rychlewski, D. Baker & N.V. Grishin: Protein structure prediction for the male-specific region of the human Y chromosome. *Proc Natl Acad Sci U S A.* 101, 2305-2310 (2004)

52. Sun C, H. Skaletsky, B. Birren, K. Devon, Z. Tang, S. Silber, R. Oates & D.C. Page: An azoospermic man with a de novo point mutation in the Y-chromosomal gene USP9Y. *Nat Genet.* 23, 429-432 (1999)

53. Foresta C, A. Ferlin & E. Moro: Deletion and expression analysis of AZFa genes on the human Y chromosome revealed a major role for DBY in male infertility. *Hum Mol Genet.* 9, 1161-1169 (2000)

54. Vinci G, F. Raicu, L. Popa, O. Popa, R. Cocos & K. McElreavey: A deletion of a novel heat shock gene on the Y chromosome associated with azoospermia. *Mol Hum Reprod.* 11, 295-298 (2005)

55. Kuroda-Kawaguchi T, H. Skaletsky, L.G. Brown, P.J. Minx, H.S. Cordum, R.H. Waterston, R.K. Wilson, S. Silber, R. Oates, S. Rozen & D.C. Page: The AZFc region of the Y chromosome features massive palindromes and uniform recurrent deletions in infertile men. *Nat Genet.* 29, 279-286 (2001)

56. Y Chromosome Consortium. A nomenclature system for the tree of human Y-chromosomal binary haplogroups. *Genome Res.* 12, 339-348 (2002)

57. Jobling MA & C. Tyler-Smith: The human Y chromosome: an evolutionary marker comes of age. *Nat Rev Genet*. 4, 598-612 (2003)

58. Jobling MA, G.A. Williams, G.A. Schiebel, G.A. Pandya, K. McElreavey, L. Salas, Rappold GA, N.A. Affara & C. Tyler-Smith: A selective difference between human Y-chromosomal DNA haplotypes. *Curr Biol.* 8, 1391-4 (1998)

59. Paracchini S, L. Stuppia, V. Gatta, G. Palka, E. Moro, C. Foresta, L. Mengua, R. Oliva, J.L. Ballesca, J.A. Kremer, R.J. van Golde, J.H. Tuerlings, T. Hargreave, A. Ross, H. Cooke, K. Huellen, P.H. Vogt & C. Tyler-Smith: Y-chromosomal DNA haplotypes in infertile European males carrying Y-microdeletions. *J Endocrinol Invest.* 23, 671-676 (2000)

60. Quintana-Murci L, C. Krausz, E. Heyer, J. Gromoll, I. Seifer, D.E Barton, T. Barrett, N.E. Skakkebaek, E. Rajpert-De Meyts, M. Mitchell, A.C. Lee, M.A. Jobling & K. McElreavey: The relationship between Y chromosome DNA haplotypes and Y chromosome deletions leading to male infertility. *Hum Genet.* 108, 55-58 (2001)

61. Krausz C & K.McElreavey: Y chromosome microdeletions in 'fertile' males. *Hum Reprod.* 16,1306-1307 (2001)

62. Almagor M, M. Dan-Goor, Y. Hovav & H. Yaffe: Spontaneous pregnancies in severe oligoasthenozoospermia. *Hum Reprod.* 6,780-781 (2001)

63. Kostiner DR, P.J. Turek & R.A. Reijo: Male infertility: analysis of the markers and genes on the human Y chromosome. *Hum Reprod*. 13, 3032-3038 (1998)

64.Thornhill AR, A.J. Guenther, G.M. Barbarotto, D.R. Session, M.A. Damario, D.A. Dumesic & K. Snow: False-positive Y-microdeletion result for a fertile male caused by an alteration under a PCR primer. *Int J Androl.* 25, 352-357 (2002)

65. Pryor JL, M. Kent-First, A. Muallem, A.H. Van Bergen, W.E. Nolten, L. Meisner & K.P. Roberts: Microdeletions in the Y chromosome of infertile men. *N Engl J Med.* 336, 534-539 (1997)

66. Chang PL, M.V. Sauer & S. Brown. Y chromosome microdeletion in a father and his four infertile sons. *Hum. Reprod.* 14, 2689-2694 (1999)

67. Saut N, P. Terriou, A. Navarro, N. Levy & M.J. Mitchell: The human Y chromosome genes BPY2, CDY1

and DAZ are not essential for sustained fertility. *Mol Hum Reprod*. 6, 789-793 (2000)

68. Krausz C, E. Rajpert-De Meyts, L. Frydelund-Larsen, L. Quintana-Murci, K. McElreavey & N.E. Skakkebaek: Double-blind Y chromosome microdeletion analysis in men with known sperm parameters and reproductive hormone profiles: microdeletions are specific for spermatogenic failure. *J Clin Endocrinol Metab*. 86, 2638-42 (2001)

69. Kunej T, B. Zorn & B. Peterlin: Y chromosome microdeletions in infertile men with cryptorchidism. Fertil Steril. 79 Suppl 3,1559-1565 (2003)

70. Frydelund-Larsen L, C. Krausz, H. Leffers, A.M. Andersson, E. Carlsen, S. Bangsboell, K. McElreavey, N.E. Skakkebaek & E. Rajpert-De Meyts: Inhibin B: a marker for the functional state of the seminiferous epithelium in patients with azoospermia factor C microdeletions. *J Clin Endocrinol Metab*. 87, 5618-5624 (2002)

71. Foresta C, A. Bettella, E. Moro, M. Rossato, M. Merico, A. Garolla & A. Ferlin: Inhibin B plasma concentrations in infertile patients with DAZ gene deletions treated with FSH. *Eur J Endocrinol.* 146, 801-806 (2002)

72. Kamp C, K. Huellen, S. Fernandes, M. Sousa, P.N. Schlegel, A. Mielnik, S. Kleiman, H. Yavetz, W. Krause, W. Kupker, R. Johannisson, W. Schulze, W. Weidner, A. Barros & P.H. Vogt: High deletion frequency of the complete AZFa sequence in men with Sertoli-cell-only syndrome. Mol *Hum Reprod.* 7, 987-994 (2001)

73. Krausz C, L. Quintana-Murci & K. McElreavey: Prognostic value of Y deletion analysis: what is the clinical prognostic value of Y chromosome microdeletion analysis? *Hum Reprod.* 15, 1431-4 (2000)

74. Warchol JB, A. Jankowska, D. Stecewicz, M. Ciesielski & R. Wasko: Analysis of the seminiferous tubules of patients with deletion of DAZ gene. *Arch Perinat Med* 6, 10-16 (2000)

75.Calogero AE, M.R. Garofalo, N. Barone, A.D. Palma, E. Vicari, R. Romeo, S. Tumino & R. D´Agata: Spontaneous regression over time of the germinal epithelium in a Y chromosome-microdeleted patient. *Hum Reprod*. 16,1845-1848 (2001)

76. Girardi SK, A. Mielnik & P.N. Schlegel. Submicroscopic deletions in the Y chromosome of infertile men. *Hum Reprod* 2,1635-1641(1997)

77. Simoni M, J. Gromoll, B. Dworniczak, C. Rolf, K. Abshagen, A. Kamischke, C. Carani, D. Meschede, H.M. Behre, J. Horst & E. Nieschlag: Screening for deletions of the Y chromosome involving the DAZ (Deleted in AZoospermia) gene in azoospermia and severe oligozoospermia. *Fertil Steril.* 67, 542-547 (1997)

78. Saut N, Terriou P, Navarro A, Levy N &Mitchell MJ: (2000) The human Y chromosome genes BPY2, CDY1 and DAZ are not essential for sustained fertility. *Mol Hum Reprod*. 6, 789-793.

79. Jaruzelska J, A. Korcz, A. Wojda, P. Jedrzejczak, J. Bierla, T. Surmacz, L. Pawelczyk D.C. Page & M. Kotecki: Mosaicism for 45,X cell line may accentuate the severity of spermatogenic defects in men with AZFc deletion. *J Med Genet*. 38, 798-802 (2001)

80. Krausz C, C. Bussani-Mastellone, S. Granchi, K. McElreavey, G. Scarselli & G. Forti: Screening for microdeletions of Y chromosome genes in patients undergoing ICSI procedure. *Hum. Reprod*. 14, 1717-1721 (1999)

81. Krausz C, L. Quintana-Murci, S. Barbaux, J.P. Siffroi, H. Rouba, D. Delafontaine, N. Souleyreau-Therville, G. Arvis, J.M. Antoine, E. Erdei, J.P. Taar, E. Tar A Jeandidier, G. Plessis, T. Bourgeron, J.P. Dadoune, M. Fellous & K. McElreavey: A high frequency of Y chromosome deletions in males with nonidiopathic infertility. *J Clin Endocrinol Metab.* 84, 3606-3612 (1999)

82. Krausz C, S. Degl'Innocenti, F. Nuti, F. Felici, M. Sansone, G. Variale & G. Forti: Natural transmission of *USP9Y* gene mutations: further insights into the role of *AZFa* genes in male fertility submitted.

83. Rossato M, A. Ferlin, A. Garolla, M. Pistorello & C. Foresta: Case report: high fertilization rate in conventional in-vitro fertilization utilizing spermatozoa from an oligozoospermic subject presenting microdeletions of the Y chromosome long arm. *Mol Hum Reprod.* 4, 473-476 (1998)

84. Silber SJ, R. Alagappan, L.G. Brown & D.C. Page: Y chromosome deletions in azoospermic and severely oligozoospermic men undergoing intracytoplasmic sperm injection after testicular sperm extraction. *Hum Reprod*. 13, 3332-3337 (1998)

85. van Golde RJ, A.M. Wetzels, R. de Graaf, J.H. Tuerlings, D.D. Braat & J.A. Kremer: Decreased fertilization rate and embryo quality after ICSI in oligozoospermic men with microdeletions in the azoospermia factor c region of the Y chromosome. *Hum Reprod.* 16, 289-292 (2001)

86. Choi JM, P. Chung, L. Veeck, A. Mielnik, G.D. Palermo & P.N. Schlegel: AZF microdeletions of the Y chromosome and in vitro fertilization outcome. *Fertil Steril.* 81, 337-341 (2004)

87. Kihaile PE, R.E. Kisanga, K. Aoki, Y. Kumasako, J. Misumi & T. Utsunomiya: Embryo outcome in Y-chromosome microdeleted infertile males after ICSI. *Mol Reprod Dev.* 68, 176-181 (2004)

88. Krausz C, G. Forti & K. McElreavey. The Y chromosome and male fertility and infertility. *Int J Androl*. 26, 70-75 (2003)

89. Oates RD, S. Silber, L.G. Brown & D.C. Page: Clinical characterization of 42 oligospermic or azoospermic men with microdeletion of the AZFc region of the Y chromosome, and of 18 children conceived via ICSI. *Hum Reprod.* 17,2813-2824 (2002)

90. Siffroi JP, C. Le Bourhis, C. Krausz, S. Barbaux, L. Quintana-Murci, S. Kanafani, H. Rouba, L. Bujan, G. Bourrouillou, I. Seifer, D. Boucher, M. Fellous, K. McElreavey & J.P. Dadoune: Sex chromosome mosaicism in males carrying Y chromosome long arm deletions.*Hum Reprod*. 15, 2559-2562 (2000)

91. Patsalis PC, C. Sismani, L. Quintana-Murci, F. Taleb-Bekkouche, C. Krausz & K. McElreavey: Effects of transmission of Y chromosome AZFc deletions. *Lancet.* 360, 1222-1224 (2002)

92. Patsalis PC, N. Skordis, C. Sismani, L. Kousoulidou, G. Koumbaris, C. Eftychi, G. Stavrides, A. Ioulianos, S. Kitsiou-Tzeli, A. Galla-Voumvouraki, Z. Kosmaidou, C.G. Hadjiathanasiou & K. McElreavey: Identification of high frequency of Y chromosome deletions in patients with sex chromosome mosaicism and correlation with the clinical phenotype and Y-chromosome instability. *Am J Med Genet* 135, 145-149 (2005)

93. Le Bourhis C, J.P. Siffroi, K. McElreavey & J.P. Dadoune: Y chromosome microdeletions and germinal mosaicism in infertile males. *Mol Hum Reprod.* 6, 688-693 (2000)

94. Brandell RA, A. Mielnik, D. Liotta, Z. Ye, L.L. Veeck, G.D. Palermo & P.N. Schlegel: AZFb deletions predict the absence of spermatozoa with testicular sperm extraction: preliminary report of a prognostic genetic test. *Hum Reprod.* 13, 2812-5 (1998)

95. Hopps CV, A. Mielnik, M. Goldstein, G.D. Palermo, Z. Rosenwaks & P.N. Schlegel: Detection of sperm in men with Y chromosome microdeletions of the AZFa, AZFb and AZFc regions. *Hum Reprod*. 18, 1660-5 (2003)

96. Repping S, C.M. Korver, R.D. Oates, S. Silber, F. Van der Veen, D.C. Page & S. Rozen: Are sequence family variants useful for identifying deletions in the human Y chromosome? *Am J Hum Genet*. 75, 514-517 (2004)

97. Machev N, N. Saut, G. Longepied, P. Terriou, A. Navarro, N. Levy, M. Guichaoua, C. Metzler-Guillemain, P. Collignon , A.M. Frances, J. Belougne, E. Clemente, J. Chiaroni, C. Chevillard, C. Durand, A. Ducourneau, N. Pech, K. McElreavey, M.G. Mattei & M.J. Mitchell: Sequence family variant loss from the AZFc interval of the human Y chromosome, but not gene copy loss, is strongly associated with male infertility. *J Med Genet*. 41, 814-825 (2004)

98. Ferlin A, A. Tessari, F. Ganz, E. Marchina, S. Barlati, A. Garolla, B. Engl & C. Foresta: Association of partial AZFc region deletions with spermatogenic impairment and male infertility. *J Med Genet*. 42, 209-213 (2005)

99. Llanos M, J. L. Ballesca, C. Gazquez, E. Margarit & R. Oliva: High frequency of gr/gr chromosome Y deletions in consecutive oligospermic ICS candidates. *Hum Reprod*. 20,216-20 (2005)

100. Hucklenbroich K, J. Gromoll, M. Heinrich, C. Hohoff, E. Nieschlag & M. Simoni: Partial deletions in the AZFc region of the Y chromosome occur in men with impaired as well as normal spermatogenesis. *Hum Reprod.* 20,191-197 (2005)

101. Giachini C, E. Guarducci, G. Longepied, S. Degl'Innocenti, L. Becherini, G. Forti, M.J. Mitchell & C. Krausz: The gr/gr deletion(s): a new genetic test in male infertility? *J Med Genet*. 42, 497-502 (2005)

102. Becherini L, E. Guarducci, S. Degl'Innocenti, M. Rotondi, G. Forti & C. Krausz: DAZL polymorphisms and susceptibility to spermatogenic failure: an example of remarkable ethnic differences. *Int J Androl*. 27, 375-381 (2004)

103. Fernandes S, K. Huellen, J. Goncalves, H. Dukal, J. Zeisler, E. Rajpert De Meyts, N.E. Skakkebaek, B. Habermann, W. Krause, M. Sousa, A. Barros & P.H. Vogt: High frequency of DAZ1/DAZ2 gene deletions in patients with severe oligozoospermia. *Mol Hum Reprod*. 8, 286-298 (2002)

104. Yen P: The fragility of fertility. *Nat Genet*. 29, 243-244 (2001)

105. Vogt PH: Genomic heterogeneity and instability of the AZF locus on the human Y chromosome. *Mol Cell Endocrinol*. 224,1-9 (2004)

106. Repping S, S.K. Van Daalen, C.M. Korver, L.G. Brown, J.D. Marszalek, J. Gianotten, R.D. Oates, S. Silber, F. Van der Veen, D.C. Page & S. Rozen: A family of human Y chromosomes has dispersed throughout northern Eurasia despite a 1.8-Mb deletion in the azoospermia factor c region. *Genomics*. 83,1046-1052 (2004)

107. Fernandes S, S. Paracchini, L.H. Meyer, G. Floridia, C. Tyler-Smith & P.H. Vogt: A large AZFc deletion removes DAZ3/DAZ4 and nearby genes from men in Y haplogroup N. *Am J Hum Genet*. 74, 180-187 (2004)

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Infertility is one of the most serious social problems facing advanced nations. In general, approximate half of all cases of infertility are caused by factors related to the male partner. To date, various treatments have been developed for male infertility and are steadily producing results. However, there is no effective treatment for patients with nonobstructive azoospermia, in which there is an absence of mature sperm in the testes.Â  The total number of patients with male infertility included in these studies has been small, fewer than 100 patients in nearly all cases.Â  3. Human Azoospermia and the Y Chromosome. In 1976, Tiepolo and Zuffardi first proposed an explanation for the role of the human Y chromosome in spermatogenesis [19].