Symposium: Genetic aspects of male (in)fertility

Azoospermia factor (AZF) in Yq11: towards a molecular understanding of its function for human male fertility and spermatogenesis

Peter Vogt was born in Zülpich, Germany in 1950. He obtained MSc and PhD degrees in biochemistry and physical chemistry respectively from the University of Aachen. In 1977, he was post-doctoral fellow first at the Max-Planck Institute of Biology in Tübingen, Germany, then at the Genetics department of the University of Nijmegen (Netherlands), learning the basic genetics of the Y chromosomal fertility genes of Drosophila. In 1982, he became an associate professor at the University of Nijmegen and extended his research on the structure and function of repetitive sequence families with a locus specific structure in Drosophila and in the human genome. He transferred his major research interest to the human Y chromosome and started in 1986 his molecular analysis on the ‘Azoospermia Factor’ (AZF) in Yq11 at the Institute of Human Genetics of the University of Heidelberg, Germany. In 2000, his research interest became more general on the molecular control mechanisms of germ cell development in humans. In 2002 he became head of the section of Molecular Genetics and Infertility at the Reproductive Medicine Centre of the University of Heidelberg. He has over 100 publications in international books and journals.

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Abstract

The Y chromosomal azoospermia factor (AZF) is essential for human spermatogenesis. It has been mapped by molecular deletion analyses to three subintervals in Yq11, AZFa, AZFb, and AZFc, containing a number of genes of which at least some control, post-transcriptionally, the RNA metabolism of other spermatogenesis genes, functionally expressed at different phases of the spermatogenic cycle. Intrachromosomal recombination events between homologous large repetitive sequence block in Yq11 are now recognized as the major cause of the AZFa, AZFb and AZFc microdeletions, and an overlap of the AZFb and AZFc regions was revealed by sequence analysis of the complete Yq11 region. The increasing knowledge of the expression patterns of AZF genes in human germ cells suggests that the DBY gene is the major AZFa gene, the RBMY gene the major AZFb gene, although a functional expression of the other AZFa/b genes in the male germ line is also most likely. Genetic redundancy might exist in AZFc because a number of gene copies in the large P1 palindrome structure in distal AZFc were found to be deleted also in fertile men.

Keywords: AZF and Yq11 microdeletions, AZF gene expressions in male germ cells, AZF gene evolution, AZF locus in Yq11, Yq11-macrodeletions and X0 cells

Macrolelitions of the Y chromosome of infertile men defines AZF locus in Yq11

The azoospermia factor (AZF) in Yq11 was first mapped by cytogenetonic observations of microscopically visible deletions (i.e. macrodelelions) of the Y chromosome in infertile patients, which always affected Yq11 (Tiepolo and Zuffardi, 1976). The six azoospermic men with a monocentric Yq11– chromosomes studied by Tiepolo and Zuffardi displayed severe disruption of spermatogenesis in their testicular tissue sections. This suggested an essential function of AZF for the cellular proliferation and differentiation of the human male germ cell. Numerous similar studies have confirmed this basic assumption (Sandberg, 1985), although the individual histological evaluation of the patients’ testicular tubules also points to an extension of the first observed phenotypes, promoting the basic idea that there might be a gradient of testicular pathologies observed with Yq11 macrodeletions, and that the AZF locus might be composed of a number of Y genes functioning along the complete spermatogenetic maturation process of the male germ cell (Faed et al., 1982; Hartung et al. 1988; Vogt, 1996).

Surprisingly, a large number of men with a macrodeletion in Yq11 were first not detectable by the routine Giemsa staining protocol because a secondary rearrangement had fused two broken Yq11– chromosomes, together forming dicentric Yq11–
chromosomes also called ‘dic-Yp’ because of the duplicated short Y arm (Daniel, 1985). Even today, these Y chromosomes are often wrongly described as being ‘normal’ because their size is similar to that of normal Y chromosome. Both can only be distinguished after staining the chromosomes with quinacline. The rearranged dic-Yp chromosomes have lost the large fluorescent heterochromatin block in Yq12, marking the normal Y chromosomes also in the interphase nucleus. The dic-Yp chromosomes are therefore also called ‘non-fluorescent’ Y chromosomes (Ynf). Today Ynf chromosomes can be easily diagnosed by FISH (fluorescence in-situ hybridization) when using, e.g. a centromeric Y specific DNA probe of the DYZ3 locus (Genome Data Base accession no. 1665101). Diagnostic is the presence of two and sometimes four FISH spots (depending on the phase arrest of the squashed chromosomes) on the patients’ metaphase Y chromosomes (Figure 1A). Identification of a Ynf chromosome in the patient’s lymphocyte nuclei can explain the occurrence of azoospermia in his testis tubules, because in all cases where a testicular biopsy was evaluated spermatogenesis was blocked before or at meiosis. Most interesting to note is the variable number of X0 nuclei in the lymphocytes of this patient group (0–80%), suggesting a rather unstable Ynf chromosome structure (Sandberg, 1985).

Obviously, Yq11 macrodeletions were not suitable to map the AZF locus in Yq11 more precisely; it was impossible to distinguish molecularly caused and chromosomally caused male infertility in these heterogenous patient groups. Most macro-Y chromosomal rearrangements in Yq11 were expected to interfere with the X–Y pairing gradient formed during leptotene. This pairing process is known to be functional for to interfere with the X–Y pairing gradient formed during macro-Y chromosomal rearrangements in Yq11 were expected male infertility in these heterogenous patient groups. Most distinguish molecularly caused and chromosomally caused variabilities for selecting those that might indeed disrupt an impossible to distinguish the observed Yq11 macro-deletions in distal Yq11, variable testicular pathology was found. In most tubules only Sertoli cells were identified, but in some tubes germ cells of different developmental stages were clearly visible and the occurrence of mature sperm cells albeit in a low number was reported from different laboratories (Kent-First et al., 1996; Reijo et al., 1996; Vogt et al., 1996; Girardi et al., 1997). Hypospermatogenesis seemed therefore to be the primary result of an AZFc deletion in distal Yq11.

This basic finding, association of the AZFa and AZFb microdeletions to a specific testicular histology, has been confirmed in numerous similar studies (Vogt, 1998; Schlegel, 2002; Krausz et al., 2003). Thus, the early prediction of a functional AZF locus in Yq11 (Tiepolo and Zuffardi, 1976) was not only confirmed by these molecular studies but also extended by subdividing AZF now structurally in three distinct spermatogenesis loci: AZFa, AZFb, AZFc

With regard to mapping the suspected Yq11 interval expressing the AZF locus, a general breakthrough only became possible after the establishment of some detailed molecular deletion maps of the human Y chromosome in different research groups. They were first based on Southern blots and the use of Y-specific probes selected from different Y-specific clone libraries (Vergnaud et al., 1986; Ferguson-Smith et al., 1987; Bardon et al., 1991; Ma et al., 1992), then on the collection of a large number of sequence tagged sites (STS) selected from large clones mapped in Yq11 (Foote et al., 1992; Vollrath et al. 1992; Kirisch et al., 1996) and application of polymerase chain reaction (PCR) technology. With these molecular tools, deletion mapping in Yq11 could be performed on a molecular scale and also with genomic DNA samples of infertile men with a normal karyotype (46,XY). The basic assumption behind the strategy to include also the 46,XY patient group in molecular deletion screening experiments was based on the general observation of high instabilities of the Ynf and Y ring chromosomes (Fryns et al., 1978; Diekmann et al., 1992), suggesting that the Yq11 chromosome structure should contain a high number of Y specific repetitive sequence blocks promoting microdeletions similar to those shown for different pericentromeric chromosome regions (Lupski, 1998). Indeed, using 14 Y-specific probes previously mapped to a molecular interval map in Yq11 by Southern blotting, different microdeletions were detected in proximal and in distal Yq11 of some men with idiopathic azoospermia (Ma et al., 1992; Vogt et al., 1992). Using a large number of STS and PCR technology, the microdeletion in distal Yq11 was then found to be most prominent in azoospermic men and in oligozoospermic men with a normal karyotype (Reijo et al., 1995, 1996). In the first large screening study with 370 individuals in the same patient groups and on the base of a detailed interval map in Yq11 (Figure 1B: 76 DNA loci), it then became possible to distinguish not only two but three different microdeletions, coined AZFa, AZFb and AZFc respectively (Vogt et al., 1996). Since the observed Yq11 microdeletions occurred as ‘de-novo’ mutation events, i.e. were restricted to the patient’s Y chromosome and not found in his family, polymorphic Y rearrangements were excluded as their origin and they could be considered as the putative molecular agents causing the men’s infertility. Most interestingly, these microdeletions interrupted spermatogenesis at three different phases: (i) a complete Sertoli-cell-only syndrome (SCO) was observed in all patients with deletion of the complete AZFa interval, i.e. only Sertoli cells, but no germ cells were visible in the tubules of their testis tissue sections; (ii) arrest at the spermatocyte stage was observed in the testicular tissue of all patients with deletion of the complete AZFb interval. The populations of spermatagonia and primary spermatocytes in all tubules analysed were in the normal range; however, no post-meiotic germ cells were identified; (iii) in patients with AZFc deletions in distal Yq11, variable testicular pathology was found. In most tubules only Sertoli cells were identified, but in some tubules germ cells of different developmental stages were clearly visible and the occurrence of mature sperm cells albeit in a low number was reported from different laboratories (Kent-First et al., 1996; Reijo et al., 1996; Vogt et al., 1996; Girardi et al., 1997). Hypospermatogenesis seemed therefore to be the primary result of an AZFc deletion in distal Yq11.

Surprisingly, it has been also repeatedly reported that this basic conclusion was wrong and that also men with AZFa and AZFb deletions present a variable testicular histology (see, e.g. Qureshi et al., 1996; Foresta et al., 1997; Najmabadi et al., 1997; Pryor et al., 1997; Luetjens et al., 2002). However, the molecular extensions of these Y deletions are most likely not comparable with that of the originally defined AZFa and AZFb deletion intervals (Vogt et al., 1996), because in cases where a re-analysis was possible, only partial AZFa and AZFb deletions have been identified (Kamp et al., 2001; Ferlin et al., 2003; Krausz et al., 2003).
A high frequency of AZFc deletions was found in men with idiopathic azoospermia (10–15%) or oligozoospermia (5–7%) in different populations (Vogt, 1998; Simoni, 2001; Krausz et al., 2003). They are therefore acknowledged as the most common known genetic lesion in men, causing nonobstructive azoospermia or severe oligozoospermia. In rare cases AZFc deletions were also found as ‘familial’ deletion events, i.e. were inherited from father to son (Vogt et al., 1996; Kleiman et al., 1999; Saut et al., 2000; Calogero et al., 2002). It has therefore been argued that AZFc is not functionally involved in maintaining male fertility, and that probably also other Y deletions would not cause a spermatogenic defect because they are simply only polymorphic variations of a functional normal Y chromosome (Pryor et al., 1997).

Indeed, if one considers the fact that many men with large deviations of the number of spermatozoa dropping below the values defined for normal male fertility by the World Health Organization (WHO: with <20 × 10⁶ per ml ejaculate) are known being able to father a child without artificial fertilization, the arguments of Pryor and colleagues sound reasonable. However, no AZFc deletion was found in genomic DNA samples of normozoospermic men, i.e. men with a sperm count above 20 × 10⁶ per ml ejaculate (Schlegel, 2002; Krausz et al., 2003), but only in DNA samples of men with a severe reduction in their sperm number (<10 × 10⁶ per ml ejaculate). In these oligozoospermic patients, AZFc deletions were found with a frequency of 5–10% and this frequency increased in men with a more severe sperm pathology like azoospermia or the oligoasthenoteratozoospermia (OAT) (see, for example, Foresta et al., 1997; Vogt, 1998; Krausz et al., 2000, 2003; Schlegel, 2002). These studies clearly show a mutation effect of AZFc deletions on the men’s spermatogenic maturation process, reducing its efficiency and causing hypospermatogenesis. Indeed, this mutation effect might not cause immediately also male infertility, because the clinical definition of male fertility and infertility is obviously different from that of normal and abnormal spermatogenesis (see above). Primarily, AZFc deletions seemed to have only a specific impact on the men’s spermatogenesis process and not on his fertility, and it can be easily explained why in rare cases men with an AZFc deletion can father a child; they are fertile because their number of spermatozoa was still sufficient for a successful fertilization of their wives’ oocytes.

It has been proposed that most likely the reduction of spermatozoa in men with an AZFc deletion is indeed the primary mutation effect and that there are secondary mutation effects which might be also age-dependent leading eventually to a complete absence of mature spermatozoa (i.e. azoospermia) in the man’s semen fluid (Vogt et al., 1996; Girardi et al., 1997; Chang et al., 1999; Saut et al., 2000). If this holds true, it would be expected that the fathers with a familial AZFc deletion described in the literature are now azoospermic. Nevertheless, one has to keep in mind that most AZFc deletions are found as ‘de-novo’ mutations, i.e. being restricted to the patient’s Y chromosome and not present on the Y chromosome of other family members. This would be not expected if the AZFc microdeletions had a polymorphic origin.

Molecular structure of the three AZF loci in Yq11

After the genomic human Y DNA sequence has been completed (Skaletzky et al., 2003), it can be shown that the long Y arm in Yq11 is indeed composed of numerous Y-specific repetitive sequence blocks (also called ‘amplicons’) as suggested earlier, and especially in the AZFc deletion interval (Kuroda-Kawaguchi et al., 2001). Most interesting, the amplicons were structurally assembled in eight palindromic structures (P1-P8) with the largest palindromic (P1: 2.9 Mb) in distal AZFc (Figure 1C). The other palindromes, P2-P8, were mapped, from distal to proximal (towards the Y centromere) in distal AZFb (P2: 246 kb; P3: 736 kb), in proximal AZFb (P4: 419 kb; P5: 996 kb), and between the AZFa and AZFb deletion intervals (P6: 266 kb; P7: 30 kb; P8: 75 kb) respectively. These eight palindromes are highly symmetrical and comprise ~25% of the complete Y-specific sequence class, that is 5.7 Mb of the genomic Yq11 sequence. Sequence analyses of the homologous amplicons mapped in the different palindromic arms revealed extensive homologies between 99.940 and 99.997% along the complete amplicon sequence (Kuroda-Kawaguchi et al., 2001; Repping et al., 2002; Skaletsky et al., 2003). They contain nine families of Y-specific protein coding genes all found to be expressed specifically in testis (Table 1). Their functional integrity is probably maintained by frequent gene conversion events between the homologous palindromic arms (Rozen et al., 2003). Assuming a mutation rate of 1.6 × 10⁻⁴ per nucleotide per year and 2.2 × 10⁻⁴ conversions per duplicate nucleotide per generation (i.e. per 20 years) it has been calculated that along the 5.4 Mb length of the eight Y palindromes (i.e. 2.7 × 10⁶ duplicated nucleotides) on average 600 duplicated nucleotides have undergone arm-to-arm gene conversion, thus distinguishing the Y chromosome of father and son (Rozen et al., 2003). If this holds true, it can be assumed that there is no one reference Y chromosome in each human population, but that the different Y chromosome lineages now combined in one pedigree (Y Chromosome Consortium, 2002) might be distinguished also by the number of AZF genes. In addition, the numerous and discontinuous single STS deletions reported earlier in Yq11 of fertile men and infertile men (Najmabadi et al., 1996; Stuppa et al., 1996; Foresta et al., 1997; Pryor et al., 1997) may be polymorphic sequence variants, not useful for any fine mapping studies of a functional AZF sequence region but for studies exploring a putative association between distinct Y chromosome haplogroups and male reproduction (McElreavey and Quitana-Murci, 2003; Fernandes et al., 2004; Repping et al., 2004).

Intrachromosomal recombination events between two repetitive specific HERV15 (Human Endogenous Retroviral #15) sequence blocks in Yq11 are now known to cause the complete AZFa deletions (Blanco et al., 2000; Kamp et al., 2000; Sun et al., 2000) and it could be shown that all the break and fusion points were located in a distance of 782 kb in two identical sequence blocks (ID1 and ID2) of the HERV15y1q and HERV15q2 sequence (Kamp et al., 2001; Figure 1B).

Local homologies between repetitive sequence blocks in the P1 and P5 palindromes in distal AZFc and proximal AZFb respectively have probably led to all AZFb deletions (Repping et al., 2002), whereas all complete AZFc deletions are caused by homologous recombinations between the 229-kb-long b2
Figure 1. (A) Cytogenetic mapping of AZF-macrodeletions with Y-specific centromere probe DYZ3 and fluorescence in-situ hybridization (FISH) method on metaphase chromosomes and interphase nuclei. On the left a monocentric Yq11– chromosome is diagnosed with one FISH spot on the metaphase Y chromosome and the interphase nucleus (see arrow); on the right, a dicentric Yp chromosome is diagnosed because of a doubled FISH spot which is also visible doubled in the interphase nucleus (see arrow). A schematically drawn picture of both Yq11 macrodeletions; the location of the DYZ3 probe is shown on the left of the FISH pictures (marked in yellow). (B) Mapping of AZF microdeletions in Yq11 interval map D1–D25 (Vogt et al., 1996) with Y specific probes and STSs. The three identified AZF regions are marked in the interval map with a specific colour code. Complete AZFa deletions have an extension of ~800 kb because always caused by recombination of the two homologous HERV15Yq1/q2 blocks located in D3 and D6 (Kamp et al., 2001). Complete AZFb deletions with an extension of 6.23 Mb overlap with AZFc deletions as indicated and are caused by recombination between large homologous repetitive sequence blocks (Repping et al., 2002) marked with the colour code of Kuroda-Kawaguchi et al. (2001). Recombination sites were mapped in P1.1 (ycl1) of the P1 palindrome and in P5.1 or P5.2 (ycl3 and ycl4) of the P5 palindrome, respectively. Similarly, AZFc deletions are always caused by recombination between the blue amplicons b2 and b4 and have an extension of 3.5 Mb. In patients with AZFb+c deletion, the proximal breakpoint was also found in the P4 palindrome and the distal breakpoint not in the b4 amplicon but in the yellow amplicon ycl2 (Repping et al., 2002). The STS that can be used to distinguish the different recombination sites of AZFb and AZFb+c and AZFc deletions are given below the amplicons. In proximal AZFb, sY1227 can be positive or negative depending on the recombination site of the AZFb deletion in ycl3 or ycl4 respectively. Similarly, marker sY1054 is positive in patients with an AZFb+c deletion but negative in patients with an AZFc deletion. For more details of the corresponding PCR-experiments see the original papers: AZFa: Kamp et al. (2001); AZFb, AZFb+c: Repping et al. (2002); AZFc: Kuroda-Kawaguchi et al. (2001). (C) The five palindromic structures P1–P5 within the Yq11 intervals D12–22 are marked with a similar colour code to that used for the corresponding amplicons and their molecular extensions are given in kb. They cover 5.3 Mb of the complete AZFb and AZFc regions.
and b4 amplicons (Kuroda-Kawaguchi et al., 2001). Interestingly, patients earlier diagnosed with deletion of the AZFb plus AZFc interval have not deleted the complete AZFc sequence but their AZFc deletion interval ends in the distal arm (P1.1) of the P1 palindrome (Figure 1B). The molecular extensions of these AZFb+cz deletions were estimated with 7.7 Mb (Repping et al., 2002).

The molecular extension of the AZFc deletion was estimated with 3.5 Mb (Kuroda-Kawaguchi et al., 2001), a value largely different from the first (0.5 Mb) given by Reijo et al. (1995) but nicely coinciding with the estimation of ~3 Mb extracted earlier from a YAC contig of the AZFc deletion interval (Kirsch et al., 1996). Most interesting, the genomic Y sequence analysis also confirmed that the AZFb interval overlaps with the proximal part of the AZFc deletion interval (Figure 1B), as was also proposed by the earlier genomic YAC contig analysis (Kirsch et al., 1996).

This raised the question whether it still makes sense to define between AZFb and AZFc additionally an AZFd deletion interval (Katagiri et al., 2004). The AZFd interval has been defined by three STS markers (Kent-First et al., 1999), one (sY145) now mapped to the distal AZFB interval and two (SY153, sY152) now mapped to the proximal AZFc interval. The sequence of sY152 is part of the repetitive intron 6 sequence in the DAZI and DAZ4 gene copies, i.e. is also located in the distal AZFc region (Fernandes et al., 2002). In summary, there is no molecular evidence for a distinct AZFd deletion interval, and also if restricted to the deletion of only sY153 as found earlier for some infertile patients (Kent-First et al., 1999), a more realistic explanation for this single marker deletion event would be that the AZFds/sY153 site represents a polymorphic deletion in proximal AZFc, since sY153 deletions were also found in some patients’ mothers (Vogt, 1998, Simoni et al., 1999).

Gene content of AZFa, AZFb, AZFc loci

The putative complete gene content of each AZF microdeletion has now been identified (Skaletsky et al., 2003). Thirty-one Y genes expressed in human testis and located in one of the AZF deletion intervals are registered. They can be subdivided into a list of 14 protein coding genes with a recognizable function because of one or more conserved functional peptide domains (Table 1) and in a list of 17 Y genes with a non-identified function because similar sequences have not yet been found in the database (Table 2). Three AZFb genes (CDY2, RPS4Y2 and XKR1) have one more copy proximal to AZFB and four AZFb genes are located in the overlapping distal AZFb/proximal AZFc interval: one copy of the Basic Protein Y 2 gene family (BPY1,2), one copy of the CDY gene (CDY1.1), two copies of the Deleted in AZoospermia gene (DAZ1, DAZ2). Only two AZFc genes, CSPG4LY, GOLGA2LY, are located in the unique part of the AZFc interval. This might explain the more severe testicular pathology associated with complete AZFc deletions when compared with that after a complete AZFb deletion. The three Y genes listed in Table 2 and designated with a CYorf symbol (CYorf74; CYorf15A; CYorf15B) are probably also encoding proteins but with unknown functions, whereas the 14 Y genes designated with a TTY (Testis specific Transcription Y) symbol are declared as non-coding genes because their exon sequences apparently have no significant open reading frame (Lahn and Page, 1997; Skaletsky et al., 2003).

Interestingly, all AZFa genes and a number of functional AZFb genes have an X chromosomal counterpart (Figure 2), and some of them are conserved also on the mouse X and Y chromosomes (Dbx/Dby, Smnc/Smyc, Rbmx/Rbmy, Uspx9/Uspxy). However, so far, their function in mouse spermatogenesis appears to be different from that in human spermatogenesis. In mouse, Uspxy is expressed in brain and testis tissue (Xu et al., 2002), whereas the human homologue USP9Y is expressed in all tissues analysed (Lahn and Page, 1997). In mouse, deletion of most Rbmy genes did not cause male infertility but only some spermatoozoa dysmorphologies (Mahadevaiah et al., 1998). In humans, for patients with AZFb deletion no germ cells were found which cross-react with the RBMY-specific antiserum (Elliot et al., 1997). This suggests an early function of the RBMY protein in human male germ cells.

The distinct spermatogenesis functions of the DBY AZFa gene, its X homologue, DBX (DDX3X) and their mouse homologues, Dby/Dbx, can be probably explained by the presence of a functional autosomal retrogene of the Dbx gene mapped on mouse chromosome 1 (PL10, D1Pas1) and with a testis specific transcription profile (Leroy et al., 1989; Session et al., 2001). A similar functional DBY retrogene has not been found in the human genome (Kim et al., 2001). The creation of intra- and retrogenes originating from their progenitor genes by recombination of their transcripts seems to be a common mechanism for male germ line genes when they are located on the sex chromosomes and have a function at meiosis (Wang, 2004). Accordingly, D1Pas1 protein is expressed predominantly in the nuclei of germ cells undergoing meiosis (Session et al., 2001), where transcription of the mouse Dby and Dbx genes is repressed because of the meiotic sex chromosome inactivation (MSCI) and formation of the sex vesicle (Fernandez-Capetillo et al., 2003). In mouse, D1Pas1 might therefore have complemented or even taken over the spermatogenic Dby gene function. In humans, an autosomal functional DBY retrogene is not necessary, since the DBY function seems to be restricted to the pre-meiotic germ cell phase and also its X homologue, DBX, is expressed predominantly first in spermatids (Ditton et al., 2004). In summary, it can be assumed that the spermatogenic functions of the human AZFa and AZFb genes and their homologous counterparts on mouse Y chromosomes are probably divergent because of a species-specific adaptation of their different germ line expression profiles, as found earlier for a number of autosomal genes functioning in the genetic network controlling male fertility (Wyckoff et al., 2000).

The AZFc locus can be considered as an evolutionarily very young spermatogenesis locus because no AZFc gene family has been found on the mouse Y chromosome. The BPY2 gene family might have evolved only recently during primate evolution. It contains three gene copies (BPY2.1–3) that were mapped with an identical exon structure (nine exons spanning ~25 kb) to the green amplicons (g1–g3). BPY2 encodes a basic protein of 13.9 kDa, is transcriptionally active in spermatids and spermatozoa, and the protein probably involved in the cytoskeletal network of microtubules formed during the
Table 1. Human Y genes with putative spermatogenesis function mapped in AZFa, AZFb, AZFc deletion intervals.

<table>
<thead>
<tr>
<th>Gene symbol</th>
<th>Gene name</th>
<th>Number of copies and code</th>
<th>Protein function homologue</th>
<th>Protein expression</th>
<th>Functional copies in Yp interval?</th>
<th>Yq11 interval</th>
<th>X chromosome homologue</th>
<th>Autosomal homologue</th>
</tr>
</thead>
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<tr>
<td><strong>BPY2</strong></td>
<td>Basic protein Y, pl 10</td>
<td>BPY2.1–3 Novel</td>
<td>Only testis</td>
<td>No</td>
<td>AZFc, proximal, AZFb, distal</td>
<td>No</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td><strong>CDY</strong></td>
<td>Chromosome domain Y</td>
<td>CDY1.1–2, CDY2.1–2 Chromatin-proteins and histone-acetyltransferase</td>
<td>Only testis</td>
<td>No</td>
<td>AZFc (CDY1), AZFb +Yq11-D11 (CDY2)</td>
<td>No</td>
<td>6p24; CDYL1, 16q23; CDYL2</td>
<td></td>
</tr>
<tr>
<td><strong>CSPG4LY</strong></td>
<td>Chondroitin sulphate, proteoglycan 4 Like Y</td>
<td>CSPG4LY.1, CSPG4LY.2 Cadherins</td>
<td>Only testis</td>
<td>No</td>
<td>AZFc</td>
<td>No</td>
<td>15q24; CSPG4</td>
<td></td>
</tr>
<tr>
<td><strong>DAZ</strong></td>
<td>Deleted in Azoospermia</td>
<td>DAZ1, DAZ2, DAZ3, DAZ4 RNA binding, RRM proteins</td>
<td>Only testis</td>
<td>No</td>
<td>AZFc, AZFb-dist.: DAZ1/2</td>
<td>No</td>
<td>3p24; DAZL1, 2q33; BOULE</td>
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<tr>
<td><strong>DBY (DDX3Y)</strong></td>
<td>DEAD box Y</td>
<td>DEAD box RNA helicases</td>
<td>Multiple</td>
<td>No</td>
<td>AZFa</td>
<td>DBX (DDX3X)</td>
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<td></td>
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<tr>
<td><strong>EIF1AY</strong></td>
<td>Essential initiation, translation factor 1A Y</td>
<td>1 Translation initiation factor</td>
<td>Multiple</td>
<td>No</td>
<td>AZFb</td>
<td>EIF1AX</td>
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<tr>
<td><strong>GOLGA2LY</strong></td>
<td>Golgi autoantigen, golgin subfamily a2 like Y</td>
<td>GOLGA2LY.1, GOLGA2LY.2 CIS GOLGI matrix protein GM130</td>
<td>Only testis</td>
<td>No</td>
<td>AZFc</td>
<td>No</td>
<td>9q34; GOLGA2</td>
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<tr>
<td><strong>HSFY</strong></td>
<td>Heat-shock transcription factor, Y linked</td>
<td>HSFY1, HSFY2 HSP-2 like</td>
<td>Testis, kidney</td>
<td>No</td>
<td>AZFb</td>
<td>No</td>
<td>6q22; HSP2</td>
<td></td>
</tr>
<tr>
<td><strong>PRY</strong></td>
<td>PTP-BL related Y</td>
<td>PRY1, PRY2 Protein tyrosine phosphatase</td>
<td>Only testis</td>
<td>Prox Yp11, pseudogenes</td>
<td>AZFb, AZFc: pseudogenes</td>
<td>No</td>
<td>No</td>
<td></td>
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<td><strong>RBMY</strong></td>
<td>RNA-binding motif Y-linked</td>
<td>RBMY1.1–6 RNA, binding RRM-proteins</td>
<td>Only testis</td>
<td>Prox. Yp11 pseudogenes</td>
<td>AZFb, AZFc: pseudogenes</td>
<td>RBMX</td>
<td>HNRP G-T retrogene</td>
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<td><strong>RPS4Y2</strong></td>
<td>Ribosomal Protein S4, Y linked 2</td>
<td>1 S4 ribosomal protein</td>
<td>Multiple</td>
<td>Distal Yp11, RPS4Y1</td>
<td>AZFb</td>
<td>RPS4X</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td><strong>SMCY</strong></td>
<td>Selected mouse C DNA Y</td>
<td>1 H–Y antigen</td>
<td>Multiple</td>
<td>No</td>
<td>AZFb</td>
<td>SMCX</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td><strong>USP9Y</strong></td>
<td>Ubiquitin-specific protease 9 Y copy</td>
<td>1 Ubiquitin-specific protease</td>
<td>Multiple</td>
<td>No</td>
<td>AZFa</td>
<td>USP9X</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td><strong>XKRY</strong></td>
<td>X–Kell blood group precursor related Y</td>
<td>XKRY.1–2 Putative membrane transport protein</td>
<td>Only testis</td>
<td>No</td>
<td>AZFb +Yq11-D11</td>
<td>No</td>
<td>No</td>
<td></td>
</tr>
</tbody>
</table>

According to Vogt et al. (1997); Kuroda-Kawaguchi et al. (2001); Repping et al. (2002); Skaletzky et al. (2003).

Additional RNA populations with smaller lengths were found only in testis tissue (Lahn and Page, 1997; Ditton et al., 2004).
### Table 2. Human Y-genes in AZFb, AZFc with unknown protein coding potential

<table>
<thead>
<tr>
<th>Gene symbol</th>
<th>Gene name</th>
<th>Protein function homologue to</th>
<th>Tissue RNA expression</th>
<th>Copies in Yp interval</th>
<th>Yq11 interval</th>
<th>X or autosome homologue</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYorf14</td>
<td>Chromosome Y, open reading frame 14</td>
<td>Unknown</td>
<td>Multiple</td>
<td>No</td>
<td>AZFb</td>
<td>X; cXorf 14</td>
</tr>
<tr>
<td>CYorf15A</td>
<td>Chromosome Y, open reading frame 15A</td>
<td>Unknown</td>
<td>Multiple</td>
<td>No</td>
<td>AZFb</td>
<td>X; cXorf 15</td>
</tr>
<tr>
<td>CYorf15B</td>
<td>Chromosome Y, open reading frame 15B</td>
<td>Unknown</td>
<td>Multiple</td>
<td>No</td>
<td>AZFb</td>
<td>X; cXorf 15</td>
</tr>
<tr>
<td>TTY1</td>
<td>Testis transcript Y1</td>
<td>No-protein encoding RNA</td>
<td>Only testis</td>
<td>Prox. Yp11</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>TTY2</td>
<td>Testis transcript Y2</td>
<td>No-protein, encoding RNA</td>
<td>Only testis</td>
<td>Prox. Yp11</td>
<td>AZFb</td>
<td>No</td>
</tr>
<tr>
<td>TTY3</td>
<td>Testis transcript Y3</td>
<td>No-protein, encoding RNA</td>
<td>Only testis</td>
<td>No</td>
<td>AZFc</td>
<td>No</td>
</tr>
<tr>
<td>TTY4</td>
<td>Testis transcript Y4</td>
<td>No-protein, encoding RNA</td>
<td>Only testis</td>
<td>No</td>
<td>AZFc</td>
<td>No</td>
</tr>
<tr>
<td>TTY5</td>
<td>Testis transcript Y5</td>
<td>No-protein, encoding RNA</td>
<td>Only testis</td>
<td>No</td>
<td>AZFb</td>
<td>No</td>
</tr>
<tr>
<td>TTY6</td>
<td>Testis transcript Y6</td>
<td>No-protein, encoding RNA</td>
<td>Only testis</td>
<td>No</td>
<td>AZFb</td>
<td>No</td>
</tr>
<tr>
<td>TTY9</td>
<td>Testis transcript Y9</td>
<td>No-protein, encoding RNA</td>
<td>Only testis</td>
<td>No</td>
<td>AZFb</td>
<td>No</td>
</tr>
<tr>
<td>TTY10</td>
<td>Testis transcript Y10</td>
<td>No-protein, encoding RNA</td>
<td>Only testis</td>
<td>No</td>
<td>AZFb</td>
<td>No</td>
</tr>
<tr>
<td>TTY12</td>
<td>Testis transcript Y12</td>
<td>No-protein, encoding RNA</td>
<td>Only testis</td>
<td>No</td>
<td>AZFb</td>
<td>No</td>
</tr>
<tr>
<td>TTY13</td>
<td>Testis transcript Y13</td>
<td>No-protein, encoding RNA</td>
<td>Only testis</td>
<td>No</td>
<td>AZFb</td>
<td>No</td>
</tr>
<tr>
<td>TTY14</td>
<td>Testis transcript Y14</td>
<td>No-protein, encoding RNA</td>
<td>Only testis</td>
<td>No</td>
<td>AZFb</td>
<td>No</td>
</tr>
<tr>
<td>TTY15</td>
<td>Testis transcript Y15</td>
<td>No-protein, encoding RNA</td>
<td>Testis, brain</td>
<td>No</td>
<td>AZFa</td>
<td>No</td>
</tr>
<tr>
<td>AZFαT1b</td>
<td>Testis transcript Y16</td>
<td>No-protein, encoding RNA</td>
<td>Only testis</td>
<td>No</td>
<td>AZFb</td>
<td>No</td>
</tr>
<tr>
<td>Phex15c</td>
<td>Testis transcript Y17</td>
<td>No-protein, encoding RNA</td>
<td>Only testis</td>
<td>No</td>
<td>AZFc</td>
<td>No</td>
</tr>
</tbody>
</table>

*aAccording to Kuroda-Kawaguchi et al. (2001); Repping et al. (2002); Skaletzky et al. (2003). Although Skaletzky et al. described 23 TTY genes (TTY1–TTY23), only the 14 listed here are mapped on the Y chromosome sequence.

*bSargent et al. (1999).

*cDitton, Hirschmann, Vogt (1999), unpublished results.

*dDegenerated by splitting and having evolved from the same ancestral CXorf15 gene.
post-meiotic elongation phase of the male germ cell (Wong et al., 2003).

The DAZ gene family has been transposed from chromosome 3 (3p24; DAZL1 locus) on to the Y chromosome after divergence of the Old World monkey line of primates, i.e. not before ~35 million years ago (Shan et al., 1996; Seboun et al., 1997). The two gene copies in proximal AZFc (DAZ1/DAZ2) form the P2 palindrome, whereas the two other gene copies (DAZ3/DAZ4) are located 1.47 Mb more distal in the centre of the large P1 palindrome in distal AZFc. In contrast to BPY2, and also the CDY gene family (see below), the exon structures of the DAZ gene copies are rather variable especially in the number of exon 7 variants also called ‘DAZ-repeat’ (Saxena et al., 2000; Fernandes et al., 2002). Most likely, the DAZ gene family has evolved and established on the Y chromosome following a duplication–diversification process similar to that proposed by Hughes (1994). It would suggest that the DAZ genes are extending or improving the functional profile of their autosomal homologues, DAZL1 on chromosome 3 and BOULE on chromosome 2 (Xu et al., 2001).

Unfortunately, it is currently not yet possible to distinguish the spermatogenetic function of the DAZ genes on the Y chromosomes from that of the autosomal DAZL1 gene. Whereas some authors proclaim an essential function of the DAZ genes in multiple cellular compartments at multiple phases of the male germ cell development (Reijo et al., 2000), others found a restriction of DAZ gene products to the post-meiotic germ cell phase with transcripts only in early spermatids and protein in late spermatids and spermatozoa (Habermann et al., 1998). Some of these discordant findings can probably be explained by the use of different polyclonal DAZ antisera, others by the use of gene probes not able to distinguish between transcripts or proteins of the DAZ or its autosomal ancestors, BOULE and DAZL1. In any case, one can assume that the complete DAZ gene family (i.e. including BOULE and DAZL1) is functional and essential for human spermatogenesis encoding different testis-specific RNA binding proteins probably involved in the translational control of transcripts of other germ line genes (Yen, 2004; see also paper by Reynolds and Cooke in this symposium). These might be as early as during fetal germ cell development, where Pumilio-2 (PUM2) proteins are functional interacting with DAZ-like proteins (Moore et al., 2003), or at meiosis, where the translation of CDC25 transcripts is probably controlled by BOULE protein (Luetjens et al., 2004), or in late spermatids where DAZL1 and DAZ proteins, both immunohistochemically identified although with different antisera (Habermann et al., 1998; Reijo et al. 2000) are functional interacting with the DAZAPI protein (Vera et al., 2002).

The CDY gene family originates from a polyadenylated mRNA of the CDYL locus on chromosome 6, which has been then retrotransposed to the Y chromosome (Lahn and Page, 1999). In contrast to its autosomal homologue expressed in all tissues analysed, the CDY gene is expressed only in testis tissue. Interestingly, the CDY1 gene copies in AZFc have evolved then further on a two-exon gene structure forming four alternative spliced transcripts encoding three different protein isoforms (Figure 3). Only the major CDY1 transcript is identical in sequence to the CDY2 transcript. All CDY isoforms are nuclear proteins with an N-terminal chromodomain probably involved in chromatin binding, and a C-terminal domain catalytic in acetylation reaction. Accordingly, CDY proteins were recently identified as histone acetyltransferases with a strong preference for histone H4 and localized in the nuclei of maturing spermatids (Lahn et al., 2002). Histone hyperacetylation in late spermatids results in a more open chromatin structure that not only facilitates the spermatogenic histone replacement, but also provides an easier access of transcriptionally regulatory proteins to the post-meiotic sperm DNA. Interestingly, the CDY autosomal homologue, CDYL, does not encode a testis specific RNA population, as found with the mouse homologue (Cdyl), but retained only its housekeeping transcripts during primate evolution. This suggests selection for functional diversification of the autosomal and Y gene copies during primate evolution, as discussed for the DAZ gene family above. Interestingly, although the CDYL gene also encodes a histone acetyltransferase, its protein sequence is only 63% homologous to that of the CDY protein (Lahn et al., 2002). This suggests that the Y gene copies in human have been evolutionarily adapted for a specific spermatogenic function.

**Figure 2.** Schematic view on location of the functional homologues of the AZFa genes, USP9Y and DBY, and AZFb genes, EIF1AY, SMCY, RBMY on the human X chromosome. The X–Y homologous genes, ZFY/ZFX and VCY/VCX, are also expressed in human testicular tissue but mapped outside the AZF intervals. Interestingly, the UTY and UTX genes are clustering with the AZFa genes respectively, their X homologous, suggesting a structural conservation of this gene triplet on both sex chromosomes.
male infertility? Both minor variants spliced out an intron sequence from the alternative spliced mRNAs published with the following GenBank accession numbers: CDY1 major-a (AF080597), CDY1 major-b (BC033041), CDY1 minor (NM004680) and CDY1 minor-short. The last variant was identified by Kleiman et al. (2001) and not deposited in the GenBank. Both minor variants spliced out an intron sequence from the CDY1 exon at two different 5′ splicing sites but with identical 3′ splicing sites. This results in an alternative two-exon structure of the CDY1 gene with a variable 5′ intron part (A) and the translation of three protein isoforms. The extensions of their coding frames are given in numbers of nucleotides without the poly(A) tail (B).

Figure 3. Schematic view of CDY1 locus in AZFc. The transcription of this CDY gene copy in human testis produces at least four alternative spliced mRNAs published with the following GenBank accession numbers: CDY1 major-a (AF080597), CDY1 major-b (BC033041), CDY1 minor (NM004680) and CDY1 minor-short. The last variant was identified by Kleiman et al. (2001) and not deposited in the GenBank. Both minor variants spliced out an intron sequence from the CDY1 exon at two different 5′ splicing sites but with identical 3′ splicing sites. This results in an alternative two-exon structure of the CDY1 gene with a variable 5′ intron part (A) and the translation of three protein isoforms. The extensions of their coding frames are given in numbers of nucleotides without the poly(A) tail (B).

Do AZF gene mutations cause male infertility? Until now, an AZF gene mutation probably associated with the occurrence of male infertility has only been found for the USP9Y gene in AZFa. After sequence analysis of the USP9Y sequence in 564 patients with azoospermia or severe oligozoospermia, a 4 bp deletion including part of the splice-donor site of exon 8, was identified as ‘de-novo’ mutation in one patient with spermatid arrest (Sun et al., 1999). This suggests a function of USP9Y during the post-meiotic phase of human spermatogenesis. However, the AZFa pathology is a complete SCO syndrome (Kamp et al., 2001), and therefore expression of the DBY gene or other genetic factors in AZFa should also contribute to the AZFa spermatogenic function. Recently, it has been shown that the ubiquitous transcribed DBY is under translational control in male germ cells (Diton et al., 2004). The DBY protein was only found in human testis tissue, and its predominant location in spermatogonia and leptotene spermatocytes suggests that it contributes functionally to the differentiation process of premeiotic male germ cells. Therefore, the DBY gene can now be considered as the major AZFa spermatogenesis gene.

In AZFb, partial proximal deletions, not including the PRY and RBMY gene loci but proven to be ‘de-novo’ deletion events, were associated with hypospermatogenesis (Ferlin et al., 2003), whereas complete AZFb deletions usually cause a complete meiotic arrest (Vogt, 1998). This suggests that the PRY and/or the RBMY gene family are the major AZFb spermatogenesis genes. Unfortunately, gene-specific ‘de-novo’ mutations have not yet been found in these gene families. However, it has been shown that both AZFb genes play a significant role during human spermatogenesis (Elliot et al., 1997; Stouffs et al., 2001, 2004).

RBMY is now known as testis-specific splicing factor. It is homologous to RBMX on the X chromosome (Figure 2), also called the HNRPN-G gene, and to a functional retrogene HNRNP G-T on chromosome 11 (11p15) predominantly expressed in pachytene spermatocytes, (Elliott et al., 2000). Expression of the RBMY protein has been shown in the nuclei of A and B spermatogonia, spermatocytes and round spermatids. This suggests a role of this AZFb gene in the nuclear metabolism of newly synthesized RNAs in the germ cell at different phases (Elliot et al., 1997; see also the paper by Ehrmann and Elliott in this symposium).

The structural and functional organization of the RBMY/RBMX/HNRPN-G-T genes in human is reminiscent of that of the Dby/Dbx/D1Pas1 genes in the mouse genome (see above); different members of the RBMY gene family have subdivided their germ line function to different spermatogenic phases and an autosomal retrogene is required for this function at meiosis. This would also explain why the lack of RBMY proteins in men with AZFb deletions cannot be compensated for by the presence of RBMX and/or HNRPN G-T proteins.

The PRY gene family in AZFb consists of two full-length copies (PRY1 and PRY2) with five exons. Pseudogene copies with deletion of the first two exons were mapped in AZFc and between AZFa and AZFb, a repeat structure of only exons 1 and 2 were found on the short and long Y arm (Stouffs et al., 2001). That means that functional PRY gene copies were only found in the AZFb region. PRY encodes a protein phosphatase probably involved in the apoptotic degradation of...
non-functional spermatozoa (Stouffs et al., 2004). In men with normal spermatogenesis, the PRY protein was found in some spermatozoa and spermatozoa but the fraction of PRY positively staining spermatozoa increased from 1.5 to 51.2% in sperm samples with increased apoptic DNA degradation visualized with the TUNEL reaction. PRY protein was not detected in premeiotic germ cells. Its contribution to the testicular pathology of men with AZFb deletion (meiotic arrest) is therefore questionable. Considering the fact that the RBMY protein is predominant in the premeiotic germ cells (Elliott et al., 1997), deletion of both gene families in AZFb patients might cause the same testicular pathology as would be found after deletion of only the RBMY genes. However, an additional spermatogenic function of the PRY genes cannot be excluded, although it is not yet known where and how PRY proteins are functionally involved in the maturation process of the post-meiotic germ cells.

Specific mutations in a gene of the AZFc gene families are difficult to map because of the expected high rate of gene conversions in the AZFc palindromes resulting in an extremely high sequence homology, >99.9% (Kuroda-Kawaguchi et al., 2001). A functional difference between genes mapped in proximal and distal AZFc respectively might be indicated by the work of Fernandes et al. (2002), who found deletions of the DAZ1/DAZ2 gene doublet only in some men with severe oligozoospermia, but DAZ3 and DAZ4 deletions also in men with normal fertility. A similar conclusion can be drawn from the work of Repping et al. (2003), who found a proximal AZFc deletion in one infertile man caused by a b1/b3 recombination event and of Stuppia et al. (1996) who reported a family in which the Y chromosome of the fertile father had a deletion in distal AZFc, which then was enlarged in the Y chromosome of his infertile son towards proximal AZFc.

**Can AZFc deletions be premutations for loss of the complete Y chromosome (X0 cells)?**

Using spermatozoa of AZFc patients in intracytoplasmic sperm injection (ICSI), 100% transmission of the AZFc deletion to the male offspring has been reported (Kent-First et al., 1996; Page et al., 1999; Oates et al., 2002). That means the sons of AZFc patients will become infertile as well. However, additionally, there seems to be a risk for AZFc offspring to develop some forms of gonadal dysgenesis or even sexual reversal as well. There is some evidence that the complete loss of the Y chromosome causing embryonic 45,X0 cells is triggered by AZFc deletions as premutations. A variable percentage of a X0 cell line was found associated with some AZFc deletions (Siffroi et al., 2000; Jaruzelska et al., 2001), as found earlier in the blood cells of patients with the Ynf or ring Y chromosomes (Sandberg, 1985). Screening the Y chromosome of 12 patients who had a 45X/46XY karyotype and presented with Turner stigmata and sexual ambiguities, a high incidence of AZFc deletions was found in this patient group (Patsalis et al., 2002). In an international survey of prenatally diagnosed 45X/46XY mosaicism, a wide spectrum of phenotypes, including Turner syndrome, mixed gonadal dysgenesis, male pseudohermaphroditism, mild mental retardation, autism, but also normal men have been reported (Chang et al., 1990). Since one individual with ambiguous genitalia, 45,X/46.XY mosaic karyotype and AZFc deletion has also been reported (Papadimas et al., 2001), it seems wise to consider seriously the general possibility of an extension of the father’s AZFc deletion in his offspring and to offer these couples preimplantation genetic diagnosis (PGD) as discussed earlier (Mansour, 2004), or at least prenatal diagnosis (PND) for revealing a mosaic 45X0/46XY-del-AZFc karyotype in the fetus as early as possible.

**AZF microdeletions are diagnostic for the absence/presence of spermatozoa in testis tubules**

The molecular analysis for complete AZF microdeletions in the Y chromosome of idiopathic azoospermic men is an attractive prognostic tool for the finding of mature spermatozoa in the patient’s testis tissue without the need to extract an RNA sample from his testicular tissue biopsy and expression analysis of a post-meiotic marker gene. Since patients with a complete AZFa or AZFb deletion usually suffer from a complete absence of germ cells (AZFa) or a complete absence of post-meiotic germ cells (AZFb), clinical testicular sperm extractions (TESE) for ICSI treatment are not recommended for these patient groups, because they are usually not successful (Schlegel, 2002; Hoppes et al., 2003; Krausz et al., 2003; Choi et al., 2004). However, if a partial AZFb, AZFc or complete AZFc deletion is diagnosed in the patient’s Y-DNA, testicular mature spermatozoa are often found (~50% of cases) at least in single testis tubules because residual local complete spermatogenesis is typical for these patient groups, eventually also resulting in the presence of some mature spermatozoa in the patient’s ejaculate (i.e. cryptozoospermia). It is, however, important to stress that if only single STS deletions have been identified in AZFa or AZFb, they must be first analysed for their molecular extensions, as described above or in the literature (Vogt et al., 1996; Simoni et al., 1999; Kamp et al., 2001; Repping et al., 2002), since only the presence of complete AZFa and AZFb deletions on the patient’s Y chromosome will predict the absence of spermatozoa in his testicular tubules.

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