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

Dr Peter H Vogt

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.

Peter H Vogt
Section of Molecular Genetics and Infertility, Department of Gynecological Endocrinology and Reproductive Medicine, University of Heidelberg, Heidelberg, Germany
Correspondence: Voßstrasse 9, D-69115 Heidelberg, Germany. Tel: +49 6221 567918/567916; Fax: +49 6221 5633710; e-mail: peter_vogt@med.uni-heidelberg.de

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*

Macrodeletions of the Y chromosome of infertile men defines *AZF* locus in Yq11

The azoospermia factor (*AZF*) in Yq11 was first mapped by cytogenetic observations of microscopically visible deletions (i.e. macrodeletions) 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 spermatogenic 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 quinacrine. 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 '*n*on-*f*luorescent' Y chromosomes (Y*nf*). Today Y*nf* 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 Y*nf* 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 Y*nf* 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 human spermatogenesis, inactivating both sex chromosomes at pachytene in a dense X–Y chromatin body ('sex vesicle'). Consequently, after some genomic heterogeneity of the Y long arm was also observed in fertile men, for a long time no-one believed in a functional *AZF* gene(s) locus, and it seemed to be impossible to distinguish the observed Yq11 macro-variabilities for selecting those that might indeed disrupt an essential *AZF* spermatogenesis gene.

AZF is subdivided into three 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; Bardoni *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; Kirsch *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 spermatogonia 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.

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* and *AZFc* (**Figure 1B**).

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 non-obstructive 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 $<20 \times 10^6$ 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 20×10^6 per ml ejaculate (Schlegel, 2002; Krausz *et al*., 2003), but only in DNA samples of men with a severe reduction in their sperm number ($<10 \times 10^6$ 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 palindrome structures (P1-P8) with the largest palindrome (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 palindrome 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 palindrome arms (Rozen *et al*., 2003). Assuming a mutation rate of 1.6×10^{-9} 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. 2.7×10^6 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; Stuppia *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 HERV15yq1 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

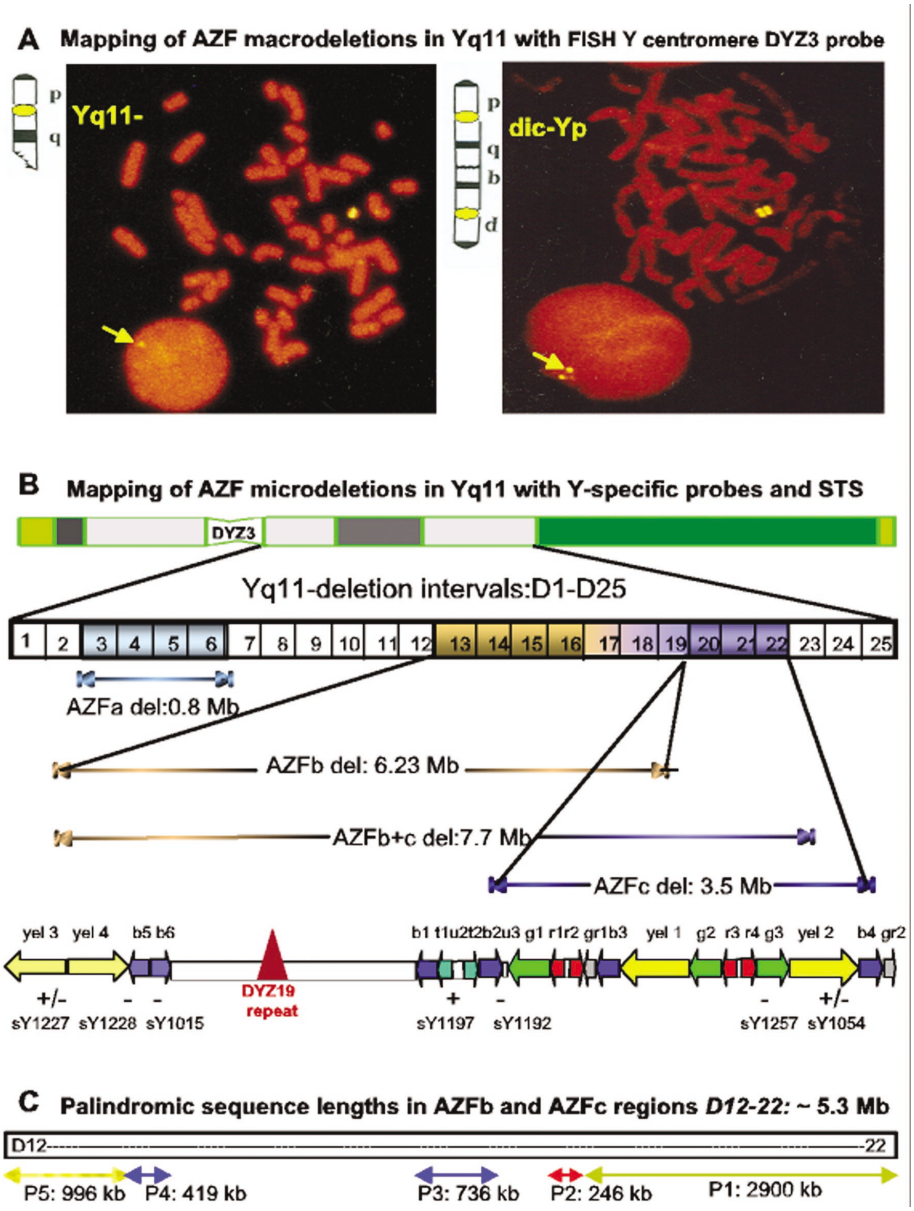

Figure 1. (**A**) Cytogenetic mapping of AZF-macrodeletions with Y-specific centromere probe DYZ3 and fluorescence in-situ hybridization (FISH) method on metaphase chromosomes and interphase nuclei. On the left a monocentric Yq11– chromosome is diagnosed with one FISH spot on the metaphase Y chromosome 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 (yel1) of the P1 palindrome and in P5.1 or P5.2 (yel3 and yel4) 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 yel2 (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 yel3 or yel4 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+c 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 region 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 *DAZ1* 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 AZFd/sY153 site represents a polymorphic deletion in proximal AZFc, since sY153 deletions were also found in some patients' fathers (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 *XKRY*) 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 (*BPY2.1*), 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 AZFb deletions when compared with that after a complete AZFc deletion. The three Y genes listed in **Table 2** and designated with a *CYorf* symbol (*CYorf14; Cyorf15A; Cyorf15B*) are probably also encoding proteins but with unknown functions, whereas the 14 Y genes designated with a *TTY* (*T*estis specific *T*ranscription *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, Smcx*/*Smcy, Rbmx*/*Rbmy, Usp9x*/*Usp9y*). However, so far, their function in mouse spermatogenesis appears to be different from that in human spermatogenesis. In mouse, *Usp9y* 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 spermatozoa 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 intron-less retrogenes originating from their progenitor genes by retroposition 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[a].

Gene symbol	*Gene name*	*Number of copies and code*	*Protein function homologue to*	*Tissue RNA expression*	*Functional copies in Yp interval?*	*Yq11 interval*	*X chromosome homologue*	*Autosomal homologue*
BPY2	Basic protein Y, pI 10	BPY2.1–3	Novel	Only testis	No	AZFc, proximal, AZFb, distal	No	No
CDY	Chromosome domain Y	CDY1.1–2, CDY2.1–2	Chromatin-proteins and histone -acetyl -transferase	Only testis	No	AZFc (CDY1), AZFb +Yq11-D11 (CDY2)	No	6p24; CDYL1, 16q23; CDYL2
CSPG4LY	Chondroitin sulphate, proteoglycan 4 Like Y	CSPG4LY.1, CSPG4LY.2	Cadherins	Only testis	No	AZFc	No	15q24; CSPG4
DAZ	Deleted in Azoospermia	DAZ1, DAZ2, DAZ3, DAZ4	RNA binding, RRM proteins	Only testis	No	AZFc, AZFb-dist.: DAZ1/2	No	3p24; DAZL1, 2q33; BOULE
DBY (*DDX3Y*)	DEAD box Y	1	DEAD box RNA helicases	Multiple[b]	No	AZFa	DBX (DDX3X	No
EIF1AY	Essential initiation, translation factor 1A Y	1	Translation initiation factor	Multiple	No	AZFb	EIF1AX	No
GOLGA2LY	Golgi autoantigen, golgin subfamily a2 like Y	GOLGA2LY.1, GOLGA2LY.2	CIS GOLGI matrix protein GM130	Only testis	No	AZFc	No	9q34; GOLGA2
HSFY	Heat-shock transcription factor, Y linked	HSFY1, HSFY2	HSP-2 like	Testis, kidney	No	AZFb	No	6q22; HSP2
PRY	PTP–BL related Y	PRY1, PRY2	Protein tyrosine phosphatase	Only testis	Prox Yp11, pseudogenes	AZFb, AZFc: pseudogenes	No	No
RBMY	RNA-binding motif Y-linked	RBMY1.1–6	RNA, binding RRM–proteins	Only testis	Prox. Yp11 pseudogenes	AZFb, AZFc: pseudogenes	RBMX	HNRNP G-T retrogene
RPS4Y2	Ribosomal Protein S4, Y linked 2	1	S4 ribosomal protein	Multiple	Distal Yp11, RPS4Y1	AZFb	RPS4X	No
SMCY	Selected mouse C DNA Y	1	H–Y antigen HLA B7	Multiple	No	AZFb	SMCX	No
USP9Y	Ubiquitin-specific protease 9 Y copy	1	Ubiquitin-specific protease	Multiple	No	AZFa	USP9X	No
XKRY	X–Kell blood group precursor related Y	XKRY.1–2	Putative membrane transport protein	Only testis	No	AZFb +Yq11-D11	No	No

[a]According to Vogt *et al*. (1997); Kuroda-Kawaguchi *et al*. (2001); Repping *et al*. (2002); Skaletzky *et al*. (2003).
[b]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[a].

Gene symbol	*Gene name*	*Protein function homologue to*	*Tissue RNA expression*	*Copies in Yp interval*	*Yq11 interval*	*X or autosome homologue*
CYorf14	Chromosome Y, open reading frame 14	Unknown	Multiple	No	AZFb	X; cXorf 14
CYorf15A[d]	Chromosome Y, open reading frame 15A	Unknown	Multiple	No	AZFb	X; cXorf 15
CYorf15B[d]	Chromosome Y, open reading frame 15B	Unknown	Multiple	No	AZFb	X; cXorf 15
TTY1	Testis transcript Y1	No-protein encoding RNA	Only testis	Prox. Yp11	No	No
TTY2	Testis transcript Y2	No-protein, encoding RNA	Only testis	Prox. Yp11	AZFb	No
TTY3	Testis transcript Y3	No-protein, encoding RNA	Only testis	No	AZFc	No
TTY4	Testis transcript Y4	No-protein, encoding RNA	Only testis	No	AZFc	No
TTY5	Testis transcript Y5	No-protein, encoding RNA	Only testis	No	AZFb	No
TTY6	Testis transcript Y6	No-protein, encoding RNA	Only testis	No	AZFb	No
TTY9	Testis transcript Y 9	No-protein, encoding RNA	Only testis	No	AZFb	No
TTY10	Testis transcript Y10	No-protein, encoding RNA	Only testis	No	AZFb	No
TTY12	Testis transcript Y12	No-protein, encoding RNA	Only testis	No	AZFb	No
TTY13	Testis transcript Y13	No-protein, encoding RNA	Only testis	No	AZFb	No
TTY14	Testis transcript Y14	No-protein, encoding RNA	Only testis	No	AZFb	No
TTY15 AZFaT1[b] Phex152[c]	Testis transcript Y15	No-protein, encoding RNA	Testis, brain	No	AZFa	No
TTY16	Testis transcript Y16	No-protein, encoding RNA	Only testis	No	AZFb	No
TTY17	Testis transcript Y17	No-protein, encoding RNA	Only testis	No	AZFc	No

[a]According 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.
[b]Sargent *et al*. (1999).
[c]Ditton, Hirschmann, Vogt (1999), unpublished results.
[d]Degenerated 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).

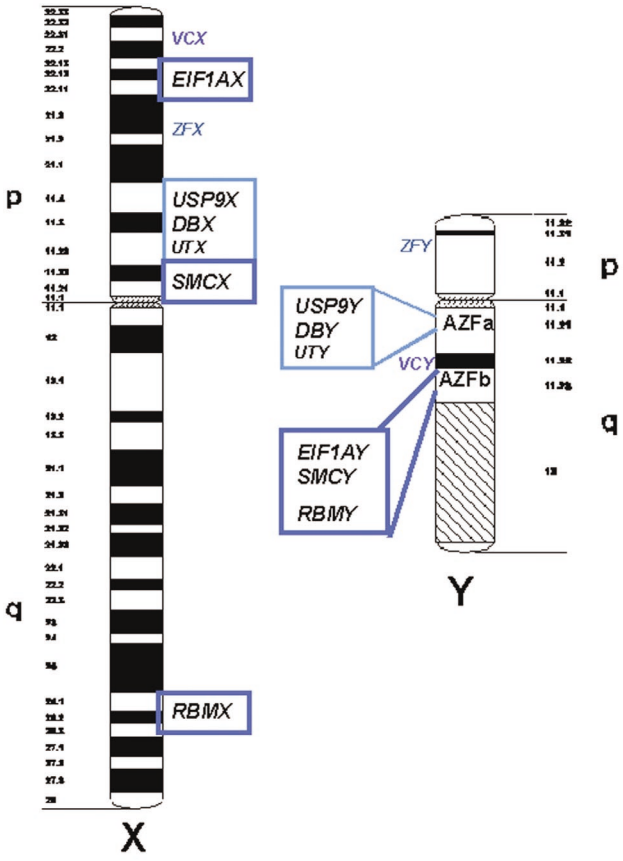

Figure 2. Schematic view on location of the functional homologues of the *AZFa* genes, *USP9Y* and *DBY*, and *AZFb* genes, *EIF1AY, SMCY, RBMY* on the human X chromosome. The X–Y homologous genes, *ZFY*/*ZFX* and *VCY*/*VCX*, are also expressed in human testicular tissue but mapped outside the AZF intervals. 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.

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 DAZAP1 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 chromo-domain 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

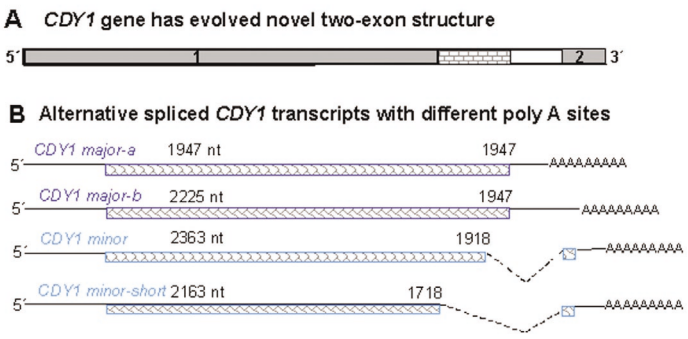

Figure 3. Schematic view of *CDY1* locus in AZFc. The transcription of this *CDY* gene copy in human testis produces at least four alternative spliced mRNAs published with the following GenBank accession numbers: *CDY1* major-a (AF080597), *CDY1* major-b (BC033041), *CDY1* minor (NM004680) and *CDY1* 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**).

(neofunctionalization) and that both genes have diverged probably already before primate evolution. In this context, it is interesting to note that a second autosomal *CDYL* gene, isolated in mouse and human (*CDYL2; Cdyl2*), both with a similar exon structure to that in the *CDYL/Cdyl* gene pair, were also divergent from each other in their amino acid sequences, as found for the *CDYL* and *CDY* genes (Dorus *et al*., 2003). The *CDY*-related gene family offers thus another informative example of how duplicated genes can evolve towards divergent functionality with a tendency to transfer genes with a spermatogenic function to the Y chromosome.

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 (Ditton *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 *HNRNP-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/HNRNP-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 HNRNP 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 spermatids 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 pre-meiotic 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 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 preimplanation 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; Hopps *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.

Acknowledgements

I would like to thank my friends and colleagues, Paul Burgoyne, David Elliott, Csilla Krausz, Richard Pfeiffer, and Peter Schlegel for their continuous and generous support of my research projects towards a molecular understanding of the function of the *AZF* locus in human spermatogenesis. My students Susana Fernandes, Christine Kamp and my technicians, Angela Edelmann, Karin Hüllen, and Jutta Zimmer, to name only some of them, are thanked for their valuable experimental contributions to the analysis of the distinct Y chromosomal rearrangements in infertile men and the *AZF* gene functions. Professor Dr Thomas Strowitzki is thanked for his continuous clinical support of the *AZF* gene project and Mrs Christine Mahrla is thanked for her extensive contributions to the final version of this manuscript.

References

Bardoni B, Zuffardi O, Guioli S *et al*. 1991 A deletion map of the human Yq11 region: implications for the evolution of the Y chromosome and tentative mapping of a locus involved in spermatogenesis. *Genomics* **11**, 443–451.

Blanco P, Shlumukova M, Sargent CA *et al*. 2000 Divergent outcomes of intrachromosomal recombination on the human Y chromosome: male infertility and recurrent polymorphism. *Journal of Medical Genetics* **37**, 752–758.

Calogero AE, Garofalo MR, Barone N *et al*. 2002 Spontaneous transmission from a father to his son of a Y chromosome microdeletion involving the deleted in azoospermia. (DAZ) gene. *Journal of Endocrinological Investigation* **25**, 631–634.

Chang HJ, Clark RD, Bachman H 1990 The phenotype of 45X/46XY mosaicism: an analysis of 92 prenatally diagnosed cases. *American Journal of Human Genetics* **46**, 156–167.

Chang PL, Sauer MV, Brown S 1999 Y chromosome microdeletion in a father and his four infertile sons. *Human Reproduction* **14**, 2689–2694.

Choi JM, Chung P, Veeck L *et al*. 2004 AZF microdeletions of the Y chromosome and in vitro fertilization outcome. *Fertility and Sterility* **81**, 337–341.

Daniel A 1985 Y isochromosomes and rings. In: Sandberg AA (ed.) *The Y Chromosome, Part B: Clinical Aspects of Y Chromosome Abnormalities*. Alan R. Liss, New York, pp. 105–135.

Diekmann L, Palm K, Pfeiffer RA *et al*. 1992 Multiple minute marker chromosomes derived from Y identified by FISH in an intersexual infant. *Human Genetics* **90**, 181–183.

Ditton HJ, Zimmer J, Kamp C *et al*. 2004 The AZFa gene DBY (DDX3Y) is widely transcribed but the protein is limited to the male germ cells by translation control. *Human Molecular Genetics* **13**, 2333–2341.

Dorus S, Gilbert SL, Forster ML *et al*. 2003 The CDY-related gene family: coordinated evolution in copy number, expression profile and protein sequence. *Human Molecular Genetics* **12**, 1643–1650.

Elliott DJ, Millar MR, Oghene K *et al*. 1997 Expression of RBM in the nuclei of human germ cells is dependent on a critical region of the Y chromosome long arm. *Proceedings of the National Academy of Sciences of the USA* **94**, 3848–3853.

Elliott DJ, Venables JP, Newton CS *et al*. 2000 An evolutionarily conserved germ cell-specific hnRNP is encoded by a retrotransposed gene. *Human Molecular Genetics* **9**, 2117–2124.

Faed MJ, Lamont MA, Baxby K 1982 Cytogenetic and histological studies of testicular biopsies from subfertile men with chromosome anomaly. *Journal of Medical Genetics* **19**, 49–56.

Ferguson-Smith MA, Affara NA, Magenis RE 1987 Ordering of Y-specific sequences by deletion mapping and analysis of X-Y interchange males and females. *Development* **101** (Suppl.), 41–50.

Ferlin A, Moro E, Rossi A *et al*. 2003 The human Y chromosome's azoospermia factor b. (AZFb) region: sequence structure and deletion analysis in infertile men. *Journal of Medical Genetics* **40**, 18–24.

Fernandes S, Huellen K, Goncalves J *et al*. 2002 High frequency of DAZ1/DAZ2 gene deletions in patients with severe oligozoospermia. *Molecular Human Reproduction* **8**, 286–298.

Fernandes S, Paracchini S, Meyer L *et al*. 2004 A large AZFc deletion removes DAZ3/DAZ4 and nearby genes from men with Y haplogroup N. *American Journal of Human Genetics* **74**, 180–187.

Fernandez-Capetillo O, Mahadevaiah K, Celeste A *et al*. 2003 H2AX is required for chromatin remodelling and inactivation of sex chromosomes in male mouse meiosis. *Developmental Cell* **4**, 497–508.

Foote S, Vollrath D, Hilton A *et al*. 1992 The human Y chromosome: overlapping DNA clones spanning the euchromatic region. *Science* **258**, 60–66.

Foresta C, Ferlin A, Garolla A *et al*. 1997 Y-chromosome deletions in idiopathic severe testiculopathies. *Journal of Clinical Endocrinology and Metabolism* **82**, 1075–1080.

Fryns JP, Cassiman JJ, Van den Berghe H 1978 Unusual in vivo rearrangements of the Y chromosome with mitotic instability in vitro. *Human Genetics* **44**, 349–355.

Girardi SK, Mielnik A, Schlegel PN 1997 Submicroscopic deletions in the Y chromosome of infertile men. *Human Reproduction* **12**, 1635–1641.

Habermann B, Mi HF, Edelmann A *et al*. 1998 DAZ. (Deleted in AZoospermia) genes encode proteins located in human late spermatids and in sperm tails. *Human Reproduction* **13**, 363–369.

Hartung M, Devictor M, Codaccioni JL *et al*. 1988 Yq deletion and failure of spermatogenesis. *Annual Genetics* **31**, 21–26.

Hopps CV, Mielnik A, Goldstein M *et al*. 2003 Detection of sperm in men with Y chromosome microdeletions of the AZFa, AZFb and AZFc regions. *Human Reproduction* **18**, 1660–1665.

Hughes AL 1994 The evolution of functionally novel proteins after gene duplication. *Proceedings of the Royal Society of London B Biological Science* **256**, 119–124.

Jaruzelska J, Korcz A, Wojda A *et al*. 2001 Mosaicism for 45X cell line may accentuate the severity of spermatogenic defects in men with AZFc deletion. *Journal of Medical Genetics* **38**, 798–802.

Kamp C, Hirschmann P, Voss H *et al*. 2000 Two long homologous retroviral sequence blocks in proximal Yq11 cause AZFa microdeletions as a result of intrachromosomal recombination events. *Human Molecular Genetics* **9**, 2563–2572.

Kamp C, Huellen K, Fernandes S *et al*. 2001 High deletion frequency of the complete AZFa sequence in men with Sertoli-cell-only syndrome. *Molecular Human Reproduction* **7**, 987–994.

Katagiri Y, Neri QV, Takeuchi T *et al*. 2004 Y chromosome assessment and its implications for the development of ICSI children. *Reproductive BioMedicine Online* **8**, 307–318.

Kent-First MG, Kol S, Muallem A *et al*. 1996 The incidence and possible relevance of Y-linked microdeletions in babies born after intracytoplasmic sperm injection and their infertile fathers. *Molecular Human Reproduction* **2**, 943–950.

Kent-First M, Muallem A, Shultz J *et al*. 1999 Defining regions of the Y-chromosome responsible for male infertility and identification of a fourth AZF region (AZFd) by Y-chromosome microdeletion detection. *Molecular Reproduction and Development* **53**, 27–41.

Kim Y-S, Lee S-G, Park SH *et al*. 2001 Gene structure of the human DDX3 and chromosome mapping of its related sequences. *Molecular Cells* **12**, 206–214.

Kirsch S, Keil R, Edelmann A *et al*. 1996 Molecular analysis of the genomic structure of the human Y chromosome in the euchromatic part of its long arm (Yq11). *Cytogenetics and Cell Genetics* **75**, 197–206.

Kleiman SE, Yogev L, Gamzu R *et al*. 1999 Three-generation evaluation of Y-chromosome microdeletion. *Journal of Andrology* **20**, 394–398.

Kleiman SE, Lagziel A, Yogev L *et al*. 2001 Expression of CDY1 may identify complete spermatogenesis. *Fertility and Sterility* **75**, 166–173.

Krausz C, Quintana-Murci L, McElreavey K 2000 Prognostic value of Y deletion analysis: what is the clinical prognostic value of Y chromosome microdeletion analysis? Human Reproduction **15**, 1431–1434.

Krausz C, Forti G, McElreavey K 2003 The Y chromosome and male fertility and infertility. *International Journal of Andrology* **26**, 70–75.

Kuroda-Kawaguchi T, Skaletzsky H, Brown LG *et al*. 2001 The AZFc region of the Y chromosome features massive palindromes and uniform recurrent deletions in infertile men. *National Genetics* **29**, 279–286.

Lahn BT, Page DC 1997 Functional coherence of the human Y chromosome. *Science* **278**, 675–679.

Lahn BT, Page DC 1999 Retroposition of autosomal mRNA yielded testis-specific gene family on human Y chromosome. *Nature Genetics* **21**, 429–433.

Lahn BT, Tang ZL, Zhou J *et al*. 2002 Previously uncharacterized histone acetyltransferases implicated in mammalian

spermatogenesis. *Proceedings of the National Academy of Sciences of the USA* **99**, 8707–8712.
Leroy P, Alzari P, Sassoon D *et al*. 1989 The protein encoded by a murine male germ cell-specific transcript is a putative ATP-dependent RNA helicase. *Cell* **57**, 549–559.
Luetjens CM, Gromoll J, Engelhardt M *et al*. 2002 Manifestation of Y-chromosomal deletions in the human testis: a morphometrical and immunohistochemical evaluation. *Human Reproduction* **17**, 2258–2266.
Luetjens CM, Xu EY, Rejo Pera RA *et al*. 2004 Association of meiotic arrest with lack of BOULE protein expression in infertile men. *Journal of Clinical Endocrinology and Metabolism* **89**, 1926–1933.
Lupski JR 1998 Genomic disorders: structural features of the genome can lead to DNA rearrangements and human disease traits. *Trends in Genetics* **14**, 417–422.
Ma K, Sharkey A, Kirsch S *et al*. 1992 Towards the molecular localisation of the AZF locus: mapping of microdeletions in azoospermic men within 14 subintervals of interval 6 of the human Y chromosome. *Human Molecular Genetics* **1**, 29–33.
Mahadevaiah SK, Odorisio T, Elliott DJ *et al*. 1998 Mouse homologues of the human AZF candidate gene RBM are expressed in spermatogonia and spermatids and map to a Y chromosome deletion interval associated with a high incidence of sperm abnormalities. *Human Molecular Genetics* **7**, 715–727.
Mansour R 2004 Preimplantation genetic diagnosis for Y linked diseases: why not? *Reproductive BioMedicine Online* **8**, 144–145.
McElreavey K, Quintana-Murci L 2003 Male reproductive function and the human Y chromosome: is selection acting on the Y? *Reproductive BioMedicine Online* **7**, 17–23.
Moore FL, Jaruzelska J, Fox MS *et al*. 2003 Human Pumilio-2 is expressed in embryonic stem cells and germ cells and interacts with DAZ (Deleted in AZoospermia) and DAZ-like proteins. *Proceedings of the National Academy of Sciences of the USA* **100**, 538–543.
Najmabadi H, Huang V, Yen P *et al*. 1996 Substantial prevalence of microdeletions of the Y-chromosome in infertile men with idiopathic azoospermia and oligozoospermia detected using a sequence-tagged site-based mapping strategy. *Journal of Clinical Endocrinology and Metabolism* **81**, 1347–1352.
Oates RD, Silber S, Brown LG *et al*. 2002 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. *Human Reproduction* **17**, 2813–2824.
Page DC, Silber S, Brown LG 1999 Men with infertility caused by AZFc deletion can produce sons by intracytoplasmic sperm injection but are likely to transmit the deletion and infertility. *Human Reproduction* **14**, 1722–1726.
Papadimas J, Goulis DG, Giannouli C *et al*. 2001 Ambiguous genitalia 45X/46XY mosaic karyotype and Y chromosome microdeletions in a 17-year-old man. *Fertility and Sterility* **76**, 1261–1263.
Patsalis PC, Sismani C, Quintana-Mursi L *et al*. 2002 Effects of transmission of Y chromosome AZFc deletions. *Lancet* **360**, 1222–1224.
Pryor JL, Kent-First M, Muallem A *et al*. 1997 Microdeletions in the Y chromosome of infertile men. *New England Journal of Medicine* **20**, 534–539.
Qureshi SJ, Ross AR, Ma K *et al*. 1996 Polymerase chain reaction screening for Y chromosome microdeletions: a first step towards the diagnosis of genetically-determined spermatogenic failure in men. *Molecular Human Reproduction* **2**, 775–779.
Reijo R, Lee TY, Salo P *et al*. 1995 Diverse spermatogenic defects in humans caused by Y chromosome deletions encompassing a novel RNA-binding protein gene. *National Genetics* **10**, 383–393.
Reijo R, Alagappan RK, Patrizio P *et al*. 1996 Severe oligozoospermia resulting from deletions of azoospermia factor gene on Y chromosome. *Lancet* **347**, 1290–1293.
Reijo RA, Dorfman DM, Slee R *et al*. 2000 DAZ family proteins exist throughout male germ cell development and transit from nucleus to cytoplasm at meiosis in humans and mice. *Biological Reproduction* **63**, 1490–1496.
Repping S, Skaletsky H, Lange J *et al*. 2002 Recombination between palindromes P5 and P1 on the human Y chromosome causes massive deletions and spermatogenic failure. *American Journal of Human Genetics* **71**, 906–922.
Repping S, Skaletsky H, Brown L *et al*. 2003 Polymorphism for a 1.6-Mb deletion of the human Y chromosome persists through balance between recurrent mutation and haploid selection. *Nature Genetics* **35**, 247–251.
Repping S, Korver CM, Oates RD *et al*. 2004 Are sequence family variants useful for identifying deletions in the human Y chromosome? *American Journal of Human Genetics* **75**, 517–519.
Rozen S, Skaletsky H, Marszalek JD *et al*. 2003 Abundant gene conversion between arms of palindromes in human and ape Y chromosomes. *Nature* **423**, 873–876.
Sandberg AA (ed.) 1985 *Clinical Aspects of Y Chromosome Abnormalities*. *Progress and Topics in Cytogenetics*, Vol. 6, part B, Alan R. Liss, New York.
Sargent CA, Boucher CA, Kirsch S et al. 1999 The critical region of overlap defining the AZFa male infertility interval of proximal Yq contains three transcribed sequences. Journal of Medical Genetics 36, 670–677.
Saut N, Terriou P, Navarro A, *et al*. 2000 The human Y chromosome genes BPY2 CDY1 and DAZ are not essential for sustained fertility. *Molecular Human Reproduction* **6**, 789–793.
Saxena R, de Vries JW, Repping S *et al*. 2000 Four DAZ genes in two clusters found in the AZFc region of the human Y chromosome. *Genomics* **67**, 256–267.
Schlegel PN 2002 The Y chromosome. *Reproduction BioMedicine Online* **5**, 22–25.
Seboun E, Barbaux S, Bourgeron T *et al*. 1997 Gene sequence localization and evolutionary conservation of DAZLA a candidate male sterility gene. *Genomics* **41**, 227–235.
Session DR, Lee GS, Wolgemuth DJ 2001 Characterization of D1Pas1, a mouse autosomal homologue of the human AZFa region DBY, as a nuclear protein in spermatogenic cells. *Fertility and Sterility* **76**, 804–811.
Shan Z, Hirschmann P, Seebacher T *et al*. 1996 A SPGY copy homologous to the mouse gene *Dazla* and the *Drosophila* gene boule is autosomal and expressed only in the human male gonad. *Human Molecular Genetics* **5**, 2005–2011.
Siffroi JP, Bourhis C, Krausz C *et al*. 2000 Sex chromosome mosaicism in males carrying Y chromosome long arm deletions. *Human Reproduction* **15**, 2559–2562.
Simoni M 2001 Molecular diagnosis of Y chromosome microdeletions in Europe: state-of-the-art and quality control. *Human Reproduction* **16**, 402–409.
Simoni M, Bakker E, Eurlings MC *et al*. 1999 Laboratory guidelines for molecular diagnosis of Y-chromosomal microdeletions. *International Journal of Andrology* **22**, 292–299.
Skaletsky H, Kuroda-Kawaguchi T, Minx PJ *et al*. 2003 The male-specific region of the human Y chromosome is a mosaic of discrete sequence classes. *Nature* **423**, 825–837.
Stouffs K, Lissens W, Van Landuyt L *et al*. 2001 Characterization of the genomic organization, localization and expression of four PRY genes (PRY1, PRY2, PRY3 and PRY4). *Molecular Human Reproduction* **7**, 603–610.
Stouffs K, Lissens W, Verheyen G *et al*. 2004 Expression pattern of the Y-linked PRY gene suggests a function in apoptosis but not in spermatogenesis. *Molecular Human Reproduction* **10**, 15–21.
Stuppia L, Calabrese G, Franchi PG *et al*. 1996 Widening of a Y-chromosome interval-6 deletion transmitted from a father to his infertile son accounts for an oligozoospermia criterial region distal to the RBM1 and DAZ genes. *American Journal of Human Genetics* **59**, 1393–1395.
Sun C, Skaletsky H, Birren B *et al*. 1999 An azoospermic man with a de novo point mutation in the Y-chromosome gene USP9Y. *National Genetics* **23**, 429–432.
Sun C, Skaletsky H, Rozen S *et al*. 2000 Deletion of azoospermia factor a (AZFa) region of human Y chromosome caused by

recombination between HERV15 proviruses. *Human Molecular Genetics* **9**, 2291–2296.
Tiepolo L, Zuffardi O 1976 Localization of factors controlling spermatogenesis in the nonfluorescent portion of the human Y chromosome long arm. *Human Genetics* **34**, 119–124.
Vera Y, Dai T, Hikim AP *et al*. 2002 Deleted in azoospermia associated protein 1 shuttles between nucleus and cytoplasm during normal germ cell maturation. *Journal of Andrology* **23**, 622–628.
Vergnaud G, Page DC, Simmler MC *et al*. 1986 A deletion map of the human Y chromosome based on DNA hybridization. *American Journal of Human Genetics* **38**, 109–124.
Vogt PH 1996 Human Y chromosome function in male germ cell development. *Advances in Developmental Biology* **4**, 191–257.
Vogt PH 1998 Human chromosome deletions in Yq11, AZF candidate genes and male infertility: history and update. *Molecular Human Reprododuction* **4**, 739–744.
Vogt PH, Edelmann A, Kirsch S *et al*. 1996 Human Y chromosome azoospermia factors (AZF) mapped to different subregions in Yq11. *Human Molecular Genetics* **5**, 933–945.
Vollrath D, Foote S, Hilton A *et al*. 1992 The human Y chromosome: a 43-interval map based on naturally occurring deletions. *Science* **258**, 52–59.
Wang PJ 2004 X chromosomes, retrogenes and their role in male reproduction. *Trends* in *Endocrinology and Metabolism* **15**, 79–83.
Wong EY, Tse JY, Yao KM *et al*. 2003 Identification and characterization of human VCY2-interacting protein: VCY2IP-1, a microtubule-associated protein-like protein. *Biological Reproduction* **70**, 775–784.
Wyckoff GJ, Wang W, Wu CI 2000 Rapid evolution of male reproduction genes in the descent of man. *Nature* **403**, 304–309.
Xu EY, Moore FL, Pera RA 2001 A gene family required for human germ cell development evolved from an ancient meiotic gene conserved in metazoans. *Proceedings of the National Academy of Sciences of the USA* **98**, 7414–7419.
Xu J, Burgoyne S, Arnold AP 2002 Sex differences in sex chromosome gene expression in mouse brain. *Human Molecular Genetics* **11**, 1409–1419.
Y Chromosome Consortium 2002 A nomenclature system for the tree of human Y-chromosomal binary haplogroups. *Genome Research* **12**, 339–348.
Yen PH 2004 Putative biological functions of the DAZ family. *International Journal of Andrology* **27**, 125–129.

Received 17 August 2004; *refereed 8 October 2004*; *accepted 11 November 2004*.