Human chromosome deletions in Yq11, AZF candidate genes and male infertility: history and update

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Human chromosome deletions in Yq11 seem to occur frequently as de novo mutation events in men with idiopathic azoospermia or severe oligozoospermia. However, the molecular extensions of these deletions are variable. They can be large and therefore visible under the microscope or small, not visible under the microscope, and containing the deletion of one or more DNA loci recently mapped in an apparently consecutive order along the Yq11 chromosome region. The results of 20 extensive microdeletion screening programmes have now corroborated the prevalence of the deletion of three non-overlapping DNA regions in proximal, middle and distal Yq11, which were designated earlier as AZFa, AZFb and AZFc. Deletions of single DNA loci were also reported, but as de novo and as polymorphic mutation events. Their clinical significance with regard to the men's infertility should therefore initially be handled with caution. Multiple Y genes expressed in human testis have now been mapped to each AZF region. At least one of them should be functional in human spermatogenesis and, if mutated, cause azoospermia. However, gene-specific mutations leading to the azoospermia phenotype have not yet been found for any of these AZF candidate genes. This might raise the question as to whether an AZF gene really exists in Yq11 or if the azoospermia phenotypes are only observed after deletion of a complete AZF region, after deletion of its complete gene content. Keywords: AZF and Yq11 deletions/AZFa, AZFb, AZFc regions/AZF diagnostics/idiopathic oligo-and azoospermia/polymorphic STS loci

Human Y chromosome deletion mapping

Human Y chromosome deletions supposed to interfere with male fertility were first analysed in the karyotypes of human male metaphase chromosomes 22 years ago (Tiepolo and Zuffardi, 1976). After staining with quinacrine, terminal deletions of the long chromosome arm in six sterile men with azoospermia were observed. Quinacrine fluorescence which usually marks the heterochromatic chromatin block in the distal part of the Y long arm (Yq12), was completely absent in these men. This indicated that their Y deletions included the total Yq12 heterochromatin block and at least part of the adjacent euchromatic part of the long Y arm (Yq11,23). Consequently, it was postulated that at least one genetic Y factor essential for male germ cell development is located in distal Yq11. It was defined as the azoospermia factor (AZF). Although the presence of an azoospermia factor in Yq11 was then confirmed by many similar studies (for review, see Sandberg, 1985), further resolution of the position of the AZF region in Yq11 did not seem to be possible, due mainly to the small size of the Y chromosome in the karyotypes of the sterile men. Linkage analysis commonly used for mapping a gene locus on autosomes could not be applied in Yq11 either, due to the lack of crossing-over events with the X chromosome in this Y region. After having cloned the first genomic Y-DNA fragments, molecular deletion mapping therefore became the chosen method for mapping AZF in Yq11. Using Southern blots, these Y fragments were mapped in a linear order (pter to qter) to the Y chromosomes of men with microscopically visible Yq11 deletions. As Y-specific DNA probes were rare in Yq11, this Y region was originally subdivided into only two intervals: 5 and 6 (Vergnaud et al., 1986). The most detailed map (14 intervals) in Yq11 was then established by Ma et al. (1992) with 39 Y-specific DNA probes. This map was sufficient to indicate for the first time that at least two AZF regions exist in Yq11 (Vogt et al., 1993).

Due to the development of polymerase chain reaction (PCR) technology, it was possible to increase the number of specific genomic Y-DNA fragments exponentially. Now, only small sequence-tagged sites (STS: oligonucleotides with 15–25 nucleotides) need to be Y-specific in order to be useful as a marker locus in Yq11. Vollrath et al. (1992) established the first STS interval map of the human Y chromosome based solely on PCR. With the use of 110 STS loci in Yq11 and a panel of 96 individuals with cytogenetically visible Y deletions, he was able to subdivide Yq11 into 23 intervals (5A–5Q; 6A–6F; according to the nomenclature introduced by Vergnaud et al. in 1986). As each order of STS loci in deletion maps depends inherently on the genomic DNA samples of the patients analysed in the laboratory, it is difficult to generate a general deletion map of the Y chromosome unless each laboratory uses the same patient panel for all mapping experiments. Therefore, two extensive deletion maps of Yq11 exist at the moment: the Vollrath map dividing Yq11 into 23 intervals (5A–6F), and the Vogt map dividing Yq11 into 25 intervals (D1–D25; Vogt et al., 1996).
Four different AZF regions exist in Yq11

Using the Vollrath map, Reijo et al. (1995) mapped the AZF region to interval 6C–6E. Initially it was thought that only one AZF region is present in Yq11. However, using the Vogt map, Vogt et al. (1996) were able to map three different AZF regions in Yq11: AZFa to interval D3–D6 (corresponding roughly to interval 5C of the Vollrath map); AZFb to interval D13–D16 (corresponding roughly to interval 5O–6B of the Vollrath map); AZFc to interval D20–D22 (corresponding roughly to interval 6C–6E of the Vollrath map). AZFc therefore corresponds to the AZF region as defined by Reijo et al. (1995).

The identification of AZFa and AZFb was solely based on the analyses of interstitial microdeletions occurring de novo in the Y chromosome of men with non-obstructive azoospermia and an apparently normal Y chromosome in their metaphase karyotypes. Testis histology of men with deletion of the AZFa region revealed the complete absence of germ cells (Sertoli cell-only syndrome); testis histology of men with deletion of the AZFb region revealed an arrest of spermatogenesis before or at meiosis (Vogt et al., 1996). This suggested that at least two different spermatogenesis genes (one in AZFa, one in AZFb) were present in the corresponding genomic Y regions.

AZFc deletions are not associated with a specific interruption phase of spermatogenesis

The testis pathology of men with deletion of the AZFc region could not be associated with a specific interruption phase of spermatogenesis. Although in the majority of cases most testis tubules were depleted for germ cells (Sertoli cell-only phenotype), complete spermatogenesis was observed in single tubules as well and a reduced number of mature spermatozoa in patients with deletions is still small. There-fore, variability in testicular phenotypes may become apparent in patients with AZFc deletions histology after more patients with deletions in these Yq11 regions have been analysed. Some evidence for this possibility can be deduced from the histological studies of Foresta et al. (1996), Najmabadi et al. (1996), Qureshi et al. (1996), and Girardi et al. (1997).

Clinical significance of single STS deletion events in Yq11

The analyses of the deletion of single STS loci in sterile men are intriguing, especially in the AZFc region. These loci were...
found to be inherited (sy153, Kent-First et al., 1996; sy207 and sy272; Pryor et al., 1997) and as de-novo mutation events (sy153; Kent-First et al., 1996; sy134, sy138, sy139, sy147, sy152, sy155, sy167, sy158; Stuppia et al., 1996; sy146 and sy153; sy150, sy220, and sy232; sy240, sy245, sy240, sy242, and sy148; Pryor et al., 1997). In some cases, multiple deletions of one or more apparently consecutive STS loci were analysed in Yq11, most of which, but not all, overlap with the AZFb and/or AZFc regions (Najmabadi et al., 1996; Stuppia et al., 1996; Foresta et al., 1997). Only one proved to be a de-novo mutation event (Stuppia et al., 1996, patient 25).

As most STS loci used for deletion analyses represent small anonymous genomic DNA fragments in Yq11, a causal relationship of single STS deletions to the men’s infertility is generally difficult to judge even when they occur as de-novo mutation events. Therefore, in all cases where is there only one STS deletion in Yq11 it might be wise to regard a causal relationship with the men’s infertility initially as questionable unless it has been shown that the deleted STS locus is part of a spermatogenesis gene structure in Yq11. However, it cannot be excluded either (at least not after it has been proved) to be a de-novo mutation event.

If in the Y chromosome of one patient multiple single STS deletions were analysed as de-novo deletion events (Stuppia et al., 1996), the occurrence of multiple rearrangements in Yq11 during meiosis of the Y chromosome of the patient’s father and/or an increased instability of the patient’s Y chromosome during early embryogenesis is indicated. In these cases it would be most interesting to perform single cell deletion analyses of the Y chromosomes in the fathers’ spermatozoa. As multiple Yq11 deletion events were not reported from each infertility clinic, one may speculate that multiple single STS deletions in Yq11 do not arise randomly in the human male population but are restricted to a number of genetically sensitive individuals. This sensitivity might be based on the presence of heterozygous mutated alleles of certain DNA repair genes or on restricted environmental effects (for further discussions see also Edwards and Bishop, 1997). On the other hand, it is obvious that at least some single STS deletions in Yq11 are polymorphic deletion events in the human male population. So sy153, sy207, and sy272 deletions are not restricted to the infertile male population as they were also found in the Y chromosomes of fertile men (sy153; O.Henegariu and P.Vogt, unpublished results; sy207, sy272; Pryor et al., 1997). The RBM2 deletion, first thought to be polymorphic and a null allele in the Japanese male population, has been analysed as a cloning-sequencing artefact (Chai et al., 1997). A causal relationship of polymorphic Y deletions to male infertility can probably be excluded (see also Jobling and Tyler-Smith, 1995; Jobling et al., 1996 for further discussions).

How many AZF regions are present in Yq11?

The results of 20 extensive microdeletion screening programmes published to date (Table I) seem to support the general concept of at least three different AZF regions in Yq11: AZFa, AZFb, and AZFc. The presence of a fourth AZF region in proximal Yq11, as suggested by Foresta et al. (1996), is difficult to judge at the moment because the same patients displayed additional deletions in AZFb and AZFc. It is interesting to note that the frequency of AZFa and AZFb deletions is distinctly lower than the frequency of AZFc deletions in most laboratories.

**Molecular origins of AZF deletion events**

Nothing is known about the origins and mechanisms of the molecular rearrangements in Yq11 preceding the different AZF deletion events. Y chromosomal rearrangements are expected to occur during meiosis, spermatid development or in the spermatozoa of the germ line of the patients’ fathers and/or during early embryogenesis (Edwards and Bishop, 1997). Estimations of the molecular extensions of the AZF regions in numerous patients using PCR multiplex interval mapping suggest similar breakpoints in Yq11 for AZFa patients (Qureshi et al., 1996, Vogt et al., 1996; Vogt, 1997), AZFb patients (Vogt et al., 1996, Girardi et al., 1997; Vogt, 1997) and AZFc patients (Reijo et al., 1995; Vogt et al., 1996, Qureshi et al., 1996; Girardi et al. 1997; van der Ven et al., 1997; Vogt, 1997). This indicates breakage hotspots in Yq11 at their borderlines. Such hotspots are frequently represented by homologous local chromosome-specific repetitive DNA blocks (Yen et al., 1987). Then deletions probably occur due to unequal intrachromosomal crossing-over events at meiosis during spermatogenesis in the father. This would explain why AZFc deletions are more frequent than AZFa and AZFb deletions, as local repetitive DNA blocks are enriched in distal Yq11 in the neighbourhood of the highly repetitive heterochromatic Yq12 region. However, to enable a more detailed examination of the origin of the AZF deletions, single cell deletion analyses in Yq11 of the spermatozoa of sterile patients and of fertile control men might be an essential prerequisite.

**Multiple potential spermatogenesis genes are located in each AZF region**

It is safe to assume that at least one functional spermatogenesis gene is located in each AZF region. However, the molecular extensions of the three AZF regions in Yq11 are large enough to encompass more than one gene locus. Using physical mapping experiments, DNA lengths were estimated to range between 1 Mb (AZFa) and 3 Mb (AZFc) (Vogt et al., 1996). Thus, it was no surprise when Lahn and Page (1997) published 12 new Y genes, most of which map to one of the proposed AZF regions. They complement the first analyses of Y genes in Yq11, YRRM/RBM for Y chromosome RNA Recognition Motif/RNA Binding Motif gene (Ma et al., 1993), DAZ, for Deleted in Azoospermia gene (Reijo et al., 1995) and SPGY, for SPPermatogenesis Gene locus on the Y (Vogt, 1996). DAZ and SPGY are now known to belong to the same gene family. Consequently DAZ was renamed as DAZ1 and SPGY as DAZ2 (Vogt et al., 1997). RBM and DAZ have multiple gene copies on the Y chromosome and encode testis-specific RNA binding proteins of the RNA Recognition Motif (RRM) class (Cooke and Elliot, 1997). DAZ has one additional gene copy on the short arm of chromosome 3 (DAZH, for DAZ Homolog;
Saxena et al., 1996, SPGYLA, for SPGY-like Autosomal, Shan et al., 1996, DAZLA, for DAZ-like Autosomal, Yen et al., 1996, Seboun et al., 1997). At the third international workshop on Y chromosome mapping it was renamed with the common code: DAZLI for DAZ-like 1 (Vogt et al., 1997).

Two other Y genes isolated earlier, SMCY, which stands for Selected Mouse cDNA on the Y (Agulnik et al., 1994) and TSPY, Testis Specific Protein Y-encoded (Arnemann et al., 1991) have now been mapped to the AZFb region (Vogt, 1996; P.Urbitsch and P.H.Vogt, unpublished results). SMCY has a homologous gene copy on the X chromosome (SMCX) and TSPY is a Y-specific gene family with most gene copies in the proximal part of the short Y arm and some copies in Yq11 outside the AZFa,b,c regions (BPY1, CDY, XKRY) or in proximal Yp (PRY, RBM, TSPY, TTY1, TTY2).

Diagnostics of AZF candidate genes
Unfortunately, diagnostic gene-mutation analyses of AZF candidate genes in men with idiopathic infertility is complicated by the fact that they are not single copy genes. CDY, DAZ, PRY, RBM, TSPY, TTY1, and TTY2 are repetitive on the Y. DBY, DFFRY, EIF1AX, SMCY, UTY have a homologous gene copy on the X chromosome. Functional analyses of the TSPY, RBM and DAZ gene families were therefore recently transposed to the protein level (TSPY, Schnieders et al., 1996; RBM, Elliot et al., 1997; DAZ, Habermann et al., 1998). TSPY proteins have been analysed in distinct subsets of spermatogonia, pointing to a function of TSPY immediately prior to the spermatogonia-to-spermatocyte transition (Schnieders et al., 1996). Alkaline phosphatase treatment experiments with testicular protein extracts suggest that the major protein (p33) can be phosphorylated. Their sequence homology to the proto-oncogene SET and the nucleosome-assembly factor NAP-1 suggests that TSPY proteins serve a function related to the proliferation of spermatogonia during human spermatogenesis (Schnieders et al., 1996).

RBM proteins are present mostly in the nuclei of premeiotic germ cells (Elliot et al., 1997). This indicates an involvement in the nuclear metabolism of newly synthesized testis RNA at the complete AZF region, after deletion of its complete gene content. Targeted gene deletion analyses of the homologous mice genes (knock-out alleles) are therefore needed to reveal the effect(s) of such gene-specific mutation events on male germ cell development. All human Y genes listed in Table II were, therefore, designated only as ‘AZF candidate genes’. AZF candidate genes are also those genes expressed specifically in human testis but mapped in Yq11 outside the AZFa,b,c regions (BPY1, CDY, XKRY) or in proximal Yp (PRY, RBM, TSPY, TTY1, TTY2).

<table>
<thead>
<tr>
<th>Gene symbol</th>
<th>Gene name</th>
<th>Protein homologue</th>
<th>Tissue expression</th>
<th>Yp intervention</th>
<th>Yq11 intervention</th>
<th>X chromosome homologue</th>
<th>Autosomal homologue</th>
</tr>
</thead>
<tbody>
<tr>
<td>BPY1</td>
<td>Basic protein Y, pl 9</td>
<td>Novel</td>
<td>Only testis</td>
<td>No</td>
<td>D8</td>
<td>3p24; DAZLI</td>
<td></td>
</tr>
<tr>
<td>BPY2</td>
<td>Basic protein Y, pl 10</td>
<td>Novel</td>
<td>Only testis</td>
<td>No</td>
<td>AZFc</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>CDY</td>
<td>Chromatin packaging protein</td>
<td>Only testis</td>
<td>No</td>
<td>D10-11; 23-24</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DAZ</td>
<td>Deleted in AZoosperma</td>
<td>RNA binding</td>
<td>Only testis</td>
<td>No</td>
<td>AZFc</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>DBY</td>
<td>DEAD Box Y</td>
<td>RNA helicases</td>
<td>Multiple</td>
<td>No</td>
<td>AZFa</td>
<td>Yes; DBX</td>
<td></td>
</tr>
<tr>
<td>DFFRY</td>
<td>Drosophila fat facets related Y</td>
<td>Ubiquitin-specific proteases</td>
<td>Multiple</td>
<td>No</td>
<td>AZFa</td>
<td>Yes; DFFRX</td>
<td></td>
</tr>
<tr>
<td>EIF1AY</td>
<td>Essential initiation-translation factor 1A Y</td>
<td>Translation initiation factor</td>
<td>Multiple</td>
<td>No</td>
<td>AZFb</td>
<td>Yes; EIF1AX</td>
<td></td>
</tr>
<tr>
<td>PRY</td>
<td>PTP-PL related Y</td>
<td>Protein tyrosin phosphatase</td>
<td>Only testis</td>
<td>Proximal Yp11</td>
<td>AZFc</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>RBM</td>
<td>RNA binding motif</td>
<td>RNA binding</td>
<td>Only testis</td>
<td>Proximal Yp11</td>
<td>AZFb, AZFc</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>SMCY</td>
<td>Selected mouse cDNA Y</td>
<td>H–Y antigen HLA B7</td>
<td>Multiple</td>
<td>No</td>
<td>AZFb</td>
<td>Yes; SMCY</td>
<td></td>
</tr>
<tr>
<td>TSPY</td>
<td>Testis-specific protein Y encoded</td>
<td>SET/NAP-1 regulated cell proliferation</td>
<td>Only testis</td>
<td>Proximal Yp11</td>
<td>AZFb</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>TTY1</td>
<td>Testis transcript Y1</td>
<td>No-protein encoded RNA</td>
<td>Only testis</td>
<td>Proximal Yp11</td>
<td>AZFc</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>TTY2</td>
<td>Testis transcript Y2</td>
<td>No-protein encoded RNA</td>
<td>Only testis</td>
<td>Proximal Yp11</td>
<td>AZFc</td>
<td>No</td>
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<tr>
<td>UTY</td>
<td>Ubiquitous transcribed Y</td>
<td>H–Y antigen HYD</td>
<td>Multiple</td>
<td>No</td>
<td>AZFa</td>
<td>Yes; UTX</td>
<td></td>
</tr>
<tr>
<td>XKRY</td>
<td>XK related Y</td>
<td>Putative membrane transport protein</td>
<td>Only testis</td>
<td>D10-11</td>
<td>No</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| Table II. Human Y AZF candidate genes |

Is there any AZF gene in Yq11?
Each Y gene expressed in testis tissue and located in Yq11 in a position overlapping one of the AZF regions is, by definition, a potential AZF gene, i.e. is suspected to induce the same pathological phenotype after mutation as observed after deletion of the corresponding AZF region. However, up to now these gene mutations have not yet been analysed. So it cannot be excluded that the sterile phenotypes observed after deletion of one of the AZF regions are only produced after deletion of the complete AZF region, after deletion of its complete gene content. Targeted gene deletion analyses of the homologous mice genes (knock-out alleles) are therefore needed to reveal the effect(s) of such gene-specific mutation events on male germ cell development. All human Y genes listed in Table II were, therefore, designated only as ‘AZF candidate genes’. AZF candidate genes are also those genes expressed specifically in human testis but mapped in Yq11 outside the AZFa,b,c regions (BPY1, CDY, XKRY) or in proximal Yp (PRY, RBM, TSPY, TTY1, TTY2).
this phase of human spermatogenesis. After deletion of AZFb these proteins are absent (Elliot et al., 1997). Therefore most functional RBM gene copies must be located in the AZFb region. Six subfamilies of the RBM gene family have been described (RBM-I to RBM-VI) but analyses of their transcripts in cDNA libraries suggest that only members of the RBM-I subfamily are actively transcribed and encode functional proteins (Chai et al., 1997). It is not yet known how many RBM-I gene copies are functional in AZFb because deletion of one RBM-I gene in AZFb has not yet been diagnosed. In mice, deletion of most RBM genes (apart from one or two transcripts) in the proximal part of the short Y arm does not seem to reduce their fertility although an increase in the rate of dysmorphic spermatozoa has been observed (Mahadevaiah et al., 1998). If the same holds true for men, partial RBM gene deletions are not expected to be analysed in the collective of azoospermic patients.

DAZ proteins seem to be present predominantly in the cytoplasm of late spermatids and in sperm tails (Habermann et al., 1998). They might therefore be functional in the translational control of mRNAs in these germ cells. Another possibility may be a structural role in the formation of the complex tail structure. In patients with deletion of AZFc, DAZ proteins are absent in sperm tails. AZFc deletions, however, do not have a distinct pathological phenotype. The testis histology of this patient class does not show a spermatid arrest as expected, assuming that DAZ proteins are functional as translational control proteins in late spermatids. Moreover, DAZ deletions do not interfere with the production of mature spermatozoa although the patients only produce them in low numbers (Table I). This suggests that DAZ proteins are not essential for the terminal differentiation of male germ cells but are required for their optimal function. A similar mutation effect was described recently for the POU protein sperm-1 in mouse spermatogenesis (Pearse et al., 1997).

Up to now, most diagnostic Yq11 screening analyses in infertility clinics are based on PCR analyses of a small or large panel of genomic STS loci in Yq11 (Table I). Their main focus is to detect a deletion in one of the proposed AZF regions. The only gene analysis usually included is done on the DAZ genes using primers spanning its RRM. Some diagnostic programmes only concentrate on screening the total deletion of DAZ genes (Mulhall et al., 1997; Vereb et al., 1997). The analyses of partial deletions of DAZ gene copies are not possible by the PCR method.

In conclusion, the knowledge of the now 15 AZF candidate genes (Table II) raised the question as to whether in future the PCR analyses for deletions of genomic STS loci in Yq11 are still sufficient to diagnose the functional activity of the human Y chromosome during spermatogenesis or whether new and additional PCR multiplex systems should be developed for the clinical routine diagnostics of AZF candidate genes.

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