

AZF deletions and Y chromosomal haplogroups: history and update based on sequence

Peter H. Vogt

Section of Molecular Genetics & Infertility, Department of Gynecological Endocrinology & Reproductive Medicine, University of Heidelberg, Heidelberg, Germany

Address for correspondence: Voßstrasse 9, D-69115 Heidelberg, Germany. E-mail: peter_vogt@med.uni-heidelberg.de

AZF deletions are genomic deletions in the euchromatic part of the long arm of the human Y chromosome (Yq11) associated with azoospermia or severe oligozoospermia. Consequently, it can be assumed that these deletions remove Y chromosomal genes required for spermatogenesis. However, these ‘classical’ or ‘complete’ AZF deletions, AZFa, AZFb and AZFc, represent only a subset of rearrangements in Yq11. With the benefit of the Y chromosome sequence, more rearrangements (deletions, duplications, inversions) inside and outside the classical AZF deletion intervals have been elucidated and intra-chromosomal non-allelic homologous recombinations (NAHRs) of repetitive sequence blocks have been identified as their major cause. These include duplications in AZFa, AZFb and AZFc and the partial AZFb and AZFc deletions of which some were summarized under the pseudonym ‘gr/gr’ deletions. At least some of these rearrangements are associated with distinct Y chromosomal haplogroups and are present with similar frequencies in fertile and infertile men. This suggests a functional redundancy of the AZFb/AZFc multi-copy genes. Alternatively, the functional contribution(s) of these genes to human spermatogenesis might be different in men of different Y haplogroups, that raises the question whether, the frequency of Y haplogroups with different AZF gene contents in distinct human populations leads to a male fertility status that varies between populations or whether, the presence of the multiple Y haplogroups implies a balancing selection via genomic deletion/amplification mechanisms.

Key words: AZF deletions and amplicons in Yq11/Y chromosomal haplogroups and lineages/Y sequence polymorphisms and AZF genetic redundancy/AZF gene content and male fertility status

Introduction

In 1976 Tiepolo and Zuffardi published the chromosome pictures of six men with a monocentric Yq11 chromosome all suffering from a severe impairment of spermatogenesis diagnosed in their testicular tissue sections (Tiepolo and Zuffardi, 1976). These Y chromosomal abnormalities were ‘*de novo*’ mutations, i.e., not observed in the Y chromosome of the patients’ father, thus no polymorphic events. The authors therefore, suggested that there must be a genetically functional AZoospermia factor (AZF) on the long arm of the human Y chromosome and since they did not assume that there is a spermatogenic function of the highly polymorphic heterochromatin domain in Yq12, they postulated that this AZF locus should be located in the distal part of the euchromatic Y long arm, i.e., in Yq11.23 (Tiepolo and Zuffardi, 1976). At that time there was no idea about the genetic content of this AZF locus. Protein encoding AZF genes, functionally expressed in human testis tissue, were not expected due to the extremely variable length of the Y chromosome in fertile men (Unnerus *et al.*, 1967) including also the euchromatic

Yq11 region (Soudek *et al.*, 1973). However, this chromosome variability was stable in family pedigrees and able to assign individuals to specific populations. A functional active AZF chromatin domain was therefore first proposed, visible by the decondensation of the Y chromosome in the nuclei of spermatogonia before it pairs with the X chromosome forming a condensed X-Y chromatin structure in the spermatocyte nuclei (Speed *et al.*, 1993; Vogt *et al.*, 1995).

Today we know that there are at least fourteen protein encoding Y genes part of the AZF locus (Skaletsky *et al.*, 2003; Vogt, 2005) and that the premeiotic pairing process of the sex chromosomes along the AZF chromatin is essential for a proper meiosis of the male germ cells (Armstrong *et al.*, 1994; Turner *et al.*, 2005). Moreover, with knowledge of the euchromatic Y sequence it is now also evident that the two faces of this chromosome, namely, being polymorphic on one site, being functional for spermatogenesis at the other site, are structurally intermingled, i.e., functional AZF genes are structurally linked to the Y specific highly polymorphic DNA regions. Accordingly, multiple

Y chromosomes have developed during human evolution distinguished now by a rooted pedigree of 153 Y chromosomal haplogroups (The Y Chromosome Consortium (YCC), 2002; Jobling and Tyler-Smith, 2003). Probably these Y structures were selected to drive the male reproductive fitness of the different human populations (Gill, 2002; Charlesworth, 2003). Molecular reasons of the dynamic Y sequence structure are mainly non-allelic homologous recombination (NAHR) of the locus-specific repetitive sequence block in distal Yp and Yq11 most of which are organised in large palindromes (Skaletsky *et al.*, 2003).

This review aims to introduce the reader to the history of *AZF* deletions and the current knowledge of their association with different polymorphic rearrangements present in distinct Y haplogroups. The data presented are updated on the platform of the first genomic Y sequence now published in the data base (http://www.ensembl.org/Homo_sapiens/mapview?chr=Y) and on the first rooted pedigree of the human Y chromosomal haplogroups (The Y Chromosome Consortium (YCC), 2002; <http://ycc.biosci.arizona.edu>).

Mapping of *AZF* deletions in Yq11

The first molecular approach narrowing the extension of the genomic region in Yq11 functional important for spermatogenesis (*AZF* region) was molecular deletion mapping. Using Y-specific DNA probes mapped in Yq11, breakpoints of genomic DNA samples of infertile men displaying a cytogenetically visible Y abnormality in Yq11 were ordered in molecular interval maps (Affara *et al.*, 1986; Vergnaud *et al.*, 1986; Oosthuizen *et al.*, 1990; Bardoni *et al.*, 1991; Ma *et al.*, 1992; O'Reilly *et al.*, 1992). Sequence analyses of numerous Y clones exhibited extensive sequence homologies between the Y and other human chromosomes especially to the X chromosome (Bishop *et al.* 1984). Thus restriction fragment length polymorphism (RFLP) probes were needed in order to mark the Y located restriction fragments unambiguously.

The different Yq11 abnormalities observed in infertile men and used for these first *AZF* mapping studies were cytogenetically visible and are summarized schematically in Figure 1. Studying the testicular histology of these Yq patients, it was suggested that *AZF* most probably has a premeiotic and post-meiotic spermatogenesis function (Vogt *et al.*, 1993). Low numbers of spermatogonia besides a normal number of Sertoli cells were most often reported in all cases with terminal Yq11 deletions and unbalanced Yq translocations (Tiepolo and Zuffardi, 1976; Yunis *et al.*, 1977; Andersson *et al.*, 1988, Hartung *et al.*, 1988; Bardoni *et al.*, 1991) whereas, the testicular histology of infertile men with a balanced Yq translocation were less severe. When infertile, their testicular histology showed disruption of spermatogenesis at meiosis or after the formation of spermatids (Faed *et al.*, 1982).

A large number of men with a gross Yq11 abnormality was not detectable by the routine Giemsa-staining protocol. In these cases the broken Yq11- chromosomes of two cell nuclei have fused together in a secondary meiotic or mitotic rearrangement forming a di-centric Yq11 chromosome ('dic-(Yp)') with the duplicated Y short arms (case B in Figure 1). This Yq11 rearrangement was frequently accompanied with loss of

the Yq11 chromosome in some nuclei leading to a mosaic 45,X0/46,X,dic(Yp) karyotype. Even today, the dic-(Yp) chromosomes are often wrongly described as being 'normal' because of the size similar to that of normal Y chromosomes (Siffroi *et al.*, 2000). However, both can easily be distinguished after staining the chromosomes with quinacrine. All dic-(Yp) chromosomes have lost the large fluorescent heterochromatin block in Yq12 marking the normal Y chromosome in metaphase and interphase nuclei. Only the normal Y is fluorescent, the dic-(Yp) chromosomes were always non-fluorescent ('nf') and were therefore called 'Ynf' chromosomes (Sandberg, 1985). Today Ynf

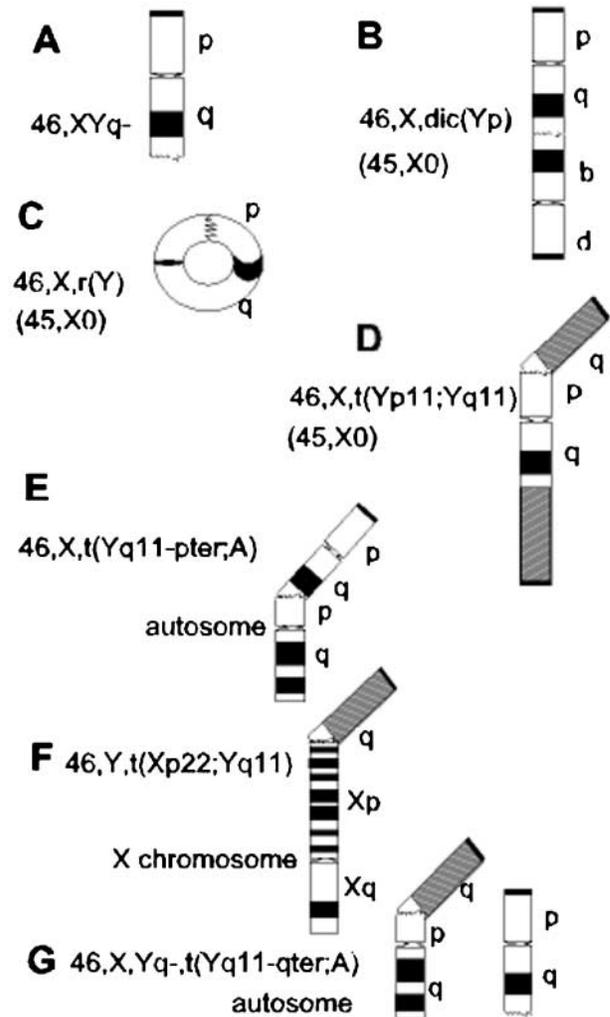


Figure 1. Schematic view on seven typical chromosomal rearrangements in Yq11 (A–G) which are associated with the occurrence of azoospermia or oligozoospermia because of disruption of the functional structure of the AZoospermia Factor (*AZF*). (A) Monocentric Yq11- chromosomes are distinguished from the normal Y chromosome by quinacrine staining or by FISH with a heterochromatin DNA probe (DYZ1; DYZ2). The same is possible with the dicentric dic-Yp chromosomes (B) and ring-Y chromosomes (C) which are often found together with a 45,X0 cell line in the patient's lymphocyte nuclei. After breakage in Yq11 and loss of the Yq11- chromosome the distal Yq11 part can also translocate to the short arm of a second Y chromosome (D), or to the short arm of the X chromosome (F). The stable Yq11- chromosome (A) can be also translocated to an autosome in a second rearrangement (E). Translocations of the distal Yq11 part to autosomes (G) are mainly to the acrocentric chromosomes: 14, 15, 21, 22 and also found in fertile men dependent on the Yq11 breakage site.

chromosomes can be easily diagnosed by fluorescence in-situ hybridization (FISH) using 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 on the patients' metaphase Y chromosomes (Vogt, 2005).

There is an inherent instability of the whole chromosome structure in Yq11 recognised by different quantities of X0 cells (1–80%) in the patient's lymphocytes and sometimes interstitial deletions in the Y short or long arm (Köhler and Vogt, 1994). The X0 cells are especially diagnosed in the lymphocytes of infertile men with a Ynf or Y-ring chromosome (Fryns *et al.*, 1978; Diekmann *et al.*, 1992; Henegariu *et al.*, 1997). Therefore, the presence of a Ynf chromosome is sometimes associated with additional pathologies: undescended testis and ambiguous genitalia (Thangaraj *et al.*, 2003) indicating the 45,X0 cells also in the patients' gonad tissues. Similar pathologies were also described from patients with an AZFc deletion and a 45,X0 cell line (Siffroi *et al.*, 2000; Jaruzelska *et al.*, 2001; Papadimas *et al.*, 2001). It suggests that Ynf chromosomes might be one of the final rearrangements of a generally unstable Y chromosome after the occurrence of a classical (i.e., complete) AZFc deletion (Vogt, 2004).

The positive identification of a Ynf chromosome in the patient's lymphocyte nuclei can explain the occurrence of azoospermia in his testis tubules because the meiotic pairing of both Yp arms (Chandley *et al.*, 1986) disrupts the pre-meiotic X-Y pairing process and meiotic silencing of the unpaired X and Y chromosomes at pachytene is inhibited (Baarends *et al.*, 2005; Turner *et al.*, 2005). Indeed, in all cases where a testicular biopsy was evaluated in this patient group spermatogenesis was blocked before or at meiosis (for review see: Vogt, 1996). Since the nature and extent of the X-Y pairing structure at the meiotic prophase temporarily involves the entire euchromatic Y long arm (Ashley, 1984; Chandley *et al.*, 1984) premeiotic 'X-Y pairing sites' in Yq11 are suggested. All cytogenetically visible Y chromosomal rearrangements in Yq11 would then interfere with the premeiotic X-Y pairing process because of the already premeiotic silencing of the unsynapsed X chromatin in the leptotene phase (Baarends *et al.*, 2005; Turner *et al.*, 2005). Consequently a chromosomally based AZF spermatogenesis function (i.e., active premeiotic 'X-Y chromosomal pairing sites' in Yq11) would be able to explain easily all cases of infertile men with cytogenetically visible Yq deletions; a meiotic disruption of spermatogenesis would be the expected main result.

Besides this proposed 'AZF-chromatin function', molecular identification of the first putative AZF genes, *RBMY* (Ma *et al.*, 1993) and *DAZ* (Reijo *et al.*, 1995), indicated that the human Y chromosome is also encoding essential protein encoding Y genes functional for spermatogenesis. At least three AZF genes should be present in Yq11 functional at different phases of the spermatogenic cycle. This was concluded from the results of a large screening program for putative AZF micro-deletions (i.e., not visible in the microscope) in 370 infertile men with a normal karyotype (46,XY) mapping three different 'de novo' deletions in Yq11 in 12 men with azoospermia or severe oligozoospermia (Vogt *et al.*, 1996). The three AZF deletion intervals designated as AZFa, AZFb, and AZFc sequence regions were confirmed in multiple similar studies (Vogt, 1998; Krausz *et al.*, 2003). These now also called

'classical' or 'complete' AZF micro-deletions are caused by intrachromosomal recombinations between homologous repetitive sequence blocks (AZFa: Blanco *et al.*, 2000; Kamp *et al.*, 2000; Sun *et al.*, 2000; AZFc: Kuroda-Kawaguchi *et al.*, 2001; AZFb: Repping *et al.*, 2002) and are always associated with the occurrence of a distinct testicular pathology (Krausz *et al.*, 2000; Krausz *et al.*, 2003; Vogt, 2005). Today we know that there are not only 3 but at least 14 protein encoding Y genes in these AZF regions (Table I).

Polymorphic Y-fragments in AZF regions establish first Y chromosomal haplogroups

If in a family pedigree, a Y-specific DNA probe detects a variant banding pattern in genomic DNA blots, it is found often to be stably inherited from father to son. This 'polymorphic' stability is probably based on the lack of regular interchromosomal recombination events (crossing overs) along the complete male specific Y sequence. Polymorphic Y sequence variants became therefore quickly established to design 'compound haplotypes' and Y chromosomal haplogroups for tracing the evolutionarily relatedness of the present human populations (Torroni *et al.*, 1990; Jobling *et al.*, 1996; Semino *et al.*, 1996). The first polymorphic sequence variants were observed with Y probes now known to map to the three 'classical' AZF deletion intervals (Figure 2).

The DYS11 DNA locus (12f2 probe; Casanova *et al.*, 1985) has been mapped to the distal HERV15 sequence block of the AZFa deletion in proximal Yq11 (Blanco *et al.*, 2000; Kamp *et al.*, 2000; Sun *et al.*, 2000). Its repetitive hybridisation pattern on genomic female DNA blots indicated that 12f2 has many related sequences on the X chromosome and on autosomes. However, the two variable fragment lengths (8, 10.4 kb) observed after *TaqI* restriction were only present in genomic male DNA, indicating their Y chromosomal origin (Figure 2). Analysis of the frequency of this 2.4 kb deletion polymorphism (here designated as DYS11 12f2-2.4 kb allele) in different populations from Europe, Africa and Asia revealed its specificity for Caucasian populations because absent in African Blacks, in Orientals and in Native Americans (Semino *et al.*, 1996). Most interesting, the frequency of the 12f2-2.4 kb deletion decreased from the Near-East to northwestern Europe populations reflecting the neolithic demic diffusion of the ancient farming cultures. Today we know that the 12f2-2.4 kb deletion is the derived state (12f2-B allele) of the undeleted ancient DYS11 sequence (12f2-A allele) and that this deletion must have occurred at least two times during evolution of the human population history (Blanco *et al.*, 2000).

The polymorphic 50f2 DNA probe (Genome Data Base accession code: DYS7: 168024) cross hybridised to five specific Y-fragments (A–E), after *EcoRI* restriction (Figure 2). 50f2/A + B was mapped to proximal Yp, 50f2/C to AZFc, 50f2/D to the Y centromer and 50f2/E to AZFb (Vogt *et al.*, 1996). The molecular base of length variation of the 50f2/B fragment in Yp is a mini-satellite block (MSY1) containing 48–114 copies of an AT rich 25 nt. long sequence unit (Jobling *et al.*, 1998). With a virtual heterozygosity of 99.9%, MSY1 is by far the most variable DNA locus on the human

Table I. Human Y genes with putative spermatogenesis function mapped to the *AZFa*, *AZFb*, *AZFc* deletion intervals^a

Gene symbol	Gene name	Number of copies and code	Protein homolog to	Tissue RNA expression	Copies in Yp interval ?	Location in Yq11	X chromosome homolog	Autosome homolog
<i>BPY2</i>	Basic Protein Y 2	<i>BPY2.1–3</i>	Novel	Only testis	no	AZFc	no	no
<i>CDY1</i>	Chromo Domain Y1/2	<i>CDY1.1–2</i>	Chromatin-Protein and histone-acetyltransferases	Only testis only	no	AZFb + Yq11-D11 (<i>CDY2</i>)	no	6p24; <i>CDYL</i>
<i>CDY2</i>		<i>CDY2.1–2</i>		testis		AZFc (<i>CDY1</i>)		
<i>CSPG4LY</i>	Chondroitin sulfate proteoglycan 4 Like Y	<i>CSPG4LY.1</i> <i>CSPG4LY.2</i>	Cadherins	Only testis	no	AZFc	no	15q24; <i>CSPG4</i>
<i>DAZ</i>	Deleted in Azoospermia	<i>DAZ1</i> , <i>DAZ2</i> , <i>DAZ3</i> , <i>DAZ4</i>	RNA binding RRM proteins	Only testis	no	AZFc	no	3p24; <i>DAZL</i> 2q33; <i>BOULE</i>
<i>DBY aka</i> <i>DDX3Y</i>	DEAD Box Y	<i>1</i>	DEAD box RNA helicases	Multiple ^b	no	AZFa	<i>DBX aka</i> <i>DDX3X</i>	no
<i>EIF1AY</i>	Essential Initiation Translat. Factor 1A Y	<i>1</i>	Translation Initiation Factor	Multiple	no	AZFb	<i>EIF1AX</i>	no
<i>GOLGA2LY</i>	Golgi autoantigen, golgin Subfamiliy a2 Like Y	<i>GOLGA2LY.1</i> <i>GOLGA2LY.2</i>	CIS GOLGI Matrix Protein GM130	Only testis	no	AZFc	no	9q34; <i>GOLGA2</i>
<i>HSFY</i>	Heat-Shock transcription Factor Y linked	<i>HSFY.1–2</i>	HSP- 2 like	Testis, kidney	no	AZFb	no	6q22; <i>HSP2</i>
<i>PRY</i>	PTP - BL Related Y	<i>PRY.1–2</i>	Protein tyrosine Phosphatase	Only testis	prox. Yp11 pseudogenes	AZFb AZFc: pseudogenes	no	no
<i>RBY</i>	RNA Binding Motif Y-linked	<i>RBY1.1–6</i>	RNA binding RRM - Proteins	Only testis	prox. Yp11 pseudogenes	AZFb AZFc: pseudogenes	<i>RBMX</i>	<i>HNRNP G-T</i> retrogene
<i>RPS4Y2</i>	Ribosomal Protein S4 Y linked 2	<i>1</i>	S4 ribosomal protein	Multiple	distal Yp11 <i>RPS4Y1</i>	AZFb	<i>RPS4X</i>	no
<i>SMCY</i>	Selected Mouse C DNA Y	<i>1</i>	H-Y antigen HLA B7	Multiple	No	AZFb	<i>SMCX</i>	no
<i>USP9Y</i>	Ubiquitin specific protease 9 Y	<i>1</i>	Ubiquitin-specific protease	Multiple	No	AZFa	<i>USP9X aka</i> <i>DFFRX</i>	no
<i>XKRY</i>	X - Kell blood group precursor related Y	<i>XKRY.1–2</i>	Putative membrane transport protein	Only testis	No	AZFb + Yq11-D11	no	no

^aAccording to Vogt (2005) with permission from Reproductive Healthcare Ltd. Data extracted from Vogt *et al.*, (1997); Kuroda-Kawaguchi *et al.*, (2001); Repping *et al.* (2002); Skaletzky *et al.*, (2003).^bAdditional RNA populations with smaller lengths were found only in testis tissue (Lahn and Page, 1997; Ditton *et al.* 2004).

Polymorphic genomic sequence patterns in the 3 AZF intervals

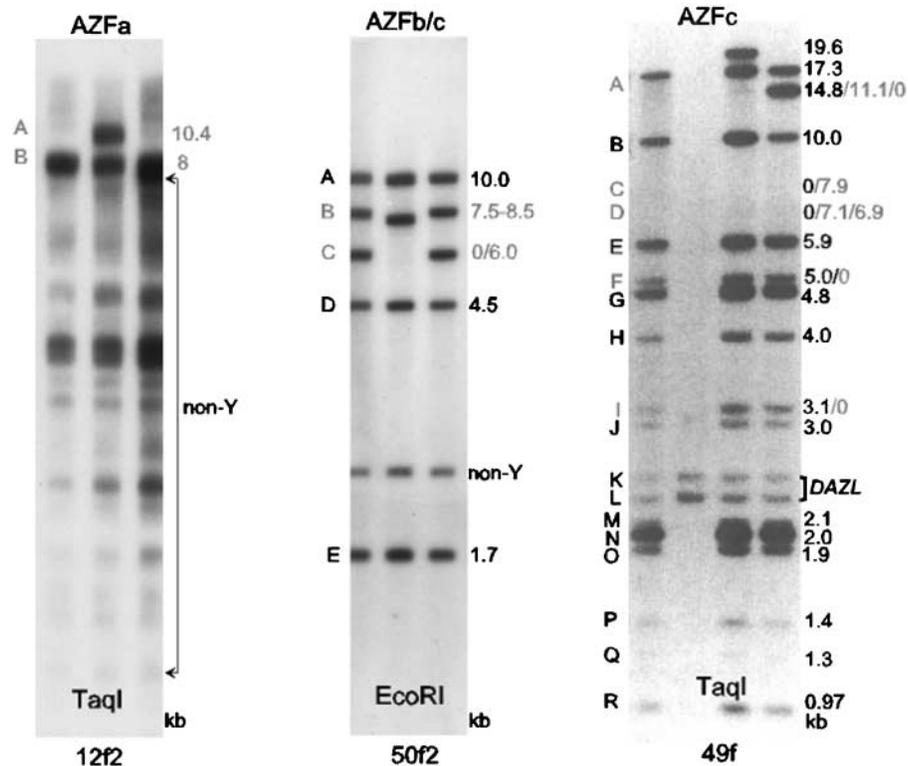


Figure 2. The first genomic sequence variants in the three AZF intervals were detected by DNA blot hybridisation with the probes 12f2 (Casanova *et al.*, 1985), 50f2 (Disteche *et al.*, 1986) and 49f (Lucotte and Ngo, 1985), respectively. The restriction enzymes used to identify the variants are given at the bottom of the blot pictures. The 12f2 probe mainly cross hybridises to a number of non-Y fragments; only the two polymorphic fragments (10.4 and 8 kb) are located on the Y chromosome. The 50f2 and 49f probes mainly cross hybridise to the Y chromosome. The 49f-K and -L *TaqI* fragments are part of the autosomal *DAZL* gene structure on the short arm of chromosome 3 (Saxena *et al.*, 1996; Vogt *et al.*, 1997), and therefore the only 49f fragments found in the female genome (see second lane from left). Letters at the left and belonging to the polymorphic fragments are coloured in grey like their expected lengths at the right of each blot picture (0 = absent). In the 49f picture, the polymorphic fragments visible in the blot are marked black in order to distinguish them from the other variants at these sites (coloured in grey; see also Table II). The 12f2 and 50f2 blots were kindly provided by Chris Tyler-Smith (The Wellcome Trust Sanger Institute, Hinxton, UK).

Y chromosome and therefore also useful for dating paternal lineages and for forensic studies (Jobling *et al.*, 1999).

After analyses of 859 men from 46 different populations absence of the 50f2/C fragment was found in 55 of them and duplications were found in 8 men (Jobling *et al.*, 1996). Additionally an independent length variation between 7.5–8.5 kb was found for the 50f2/B fragment in different individuals (Jobling *et al.*, 1998; see also Figure 2). With knowledge of the Y-sequence, 50f2/C was mapped in the u3-marked sequence block of proximal AZFc. The 50f2/E fragment was mapped proximal to it with a distance of ~1.4 Mb in AZFb (Figure 3). Since some 50f2/C deletions were associated with deletions of neighboured *RBMY* gene copies (Jobling *et al.*, 1996), these polymorphic AZFc deletions should include at least part of the AZFc t-amplicons and the b2 amplicon, i.e. would have an extension of ~500 kb (Figure 3). However, most polymorphic 50f2/C deletions were reported to be smaller not including other known DNA loci (Jobling *et al.*, 1996). The frequencies of 50f2/C deletions are variable in the 12 human populations analysed and taking their different Y chromosomal haplogroups into account, six independent deletion events and four duplication events were identified (Jobling *et al.*, 1996). Thus mul-

tipole founder rearrangements must thus have occurred during human evolution in the proximal AZFc sequence region which include all the 50f2/C *EcoRI* fragment. The highest frequency (55%) of 50f2/C deletions was found in Fins (11 of 20 men had this deletion) making it unlikely that this deletion was associated with spermatogenic failure at least in this population.

The third polymorphic AZF-locus (DYS1; 49f probe; Lucotte and Ngo, 1985) is composed of 18 *TaqI* restricted genomic Y-DNA fragments (A–R; Figure 2) and part of the *DAZ* gene structure in AZFc (Figure 3A). The variability of six *TaqI* fragments (A, C, D, F and I: present or absent; A and D modified in length: A1 = 11.1 kb; A2 = 14.8 kb; A3 = 17.3 kb; A4 = 19.6 kb; D1 = 7.2 kb; D2 = 6.9 kb) seemed to occur independently from each other and therefore established the first multi-allelic Y chromosome marker system (Ngo *et al.*, 1986). The *DYS1* length variabilities are reflecting the variable number and sequence variants of the repetitive exon 7 copies in the four *DAZ* genes (also called *DAZ*-repeats) to which the 49f probe cross hybridised; the cross hybridising K and L female fragments belong to the autosomal *DAZL* gene copy on the short arm of chromosome 3 (Saxena *et al.*, 1996; Vogt *et al.*, 1997).

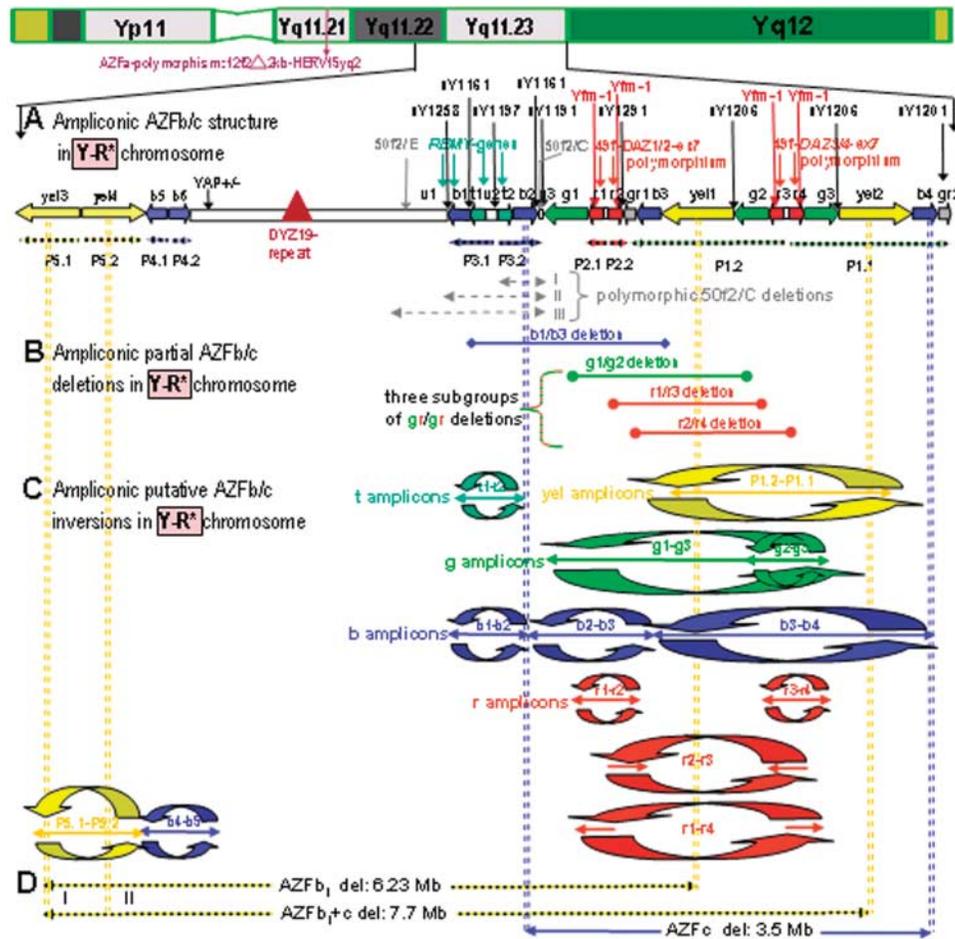


Figure 3. Schematic view on some possible variations of the AZFb/c amplicon structure in distal Yq11. (A) Structural organisation of the different amplicons in the Y chromosome of the R* haplogroup in five palindromic structures (P1–P5). The amplicons' colour code and nomenclature is derived from the work of Skaletsky *et al.* (2003). The polymorphic 49f-sites (DYS1 DNA locus) in the DAZ exon 7 repeats and the polymorphic Yfm1 marker, 25–30 kb distal to the DAZ genes (Ewis *et al.*, 2002), are located in the red amplicons. The polymorphic 50f2-DNA-locus (DYS7) is located in the AZFc-u3 region and marked with the putative extensions of its deletions in grey colour. Some 50f2/C deletions include the deletion of neighboured RBMY gene copies, some the deletion of the 50f2/E sequence site, i.e., have a size of up to 4 Mb (Jobling *et al.*, 1996; Jobling and Tyler-Smith, 2003). The polymorphic YAP deletion site (+/–) is marked distal to the P4 amplicon in AZFb. The location of the 12f2-2kb deletion polymorphism in distal HERV15yq2 of the AZFa deletion interval is marked in Yq11.21. STS markers selected for the detection of partial and total AZFb/c deletions in the Y–R* chromosome (Repping *et al.*, 2003) are given in black above the AZFb/c amplicon structure. (B) Schematic view on the extensions of possible partial AZFb/c deletions in the Y–R* chromosome. All are based on the assumption that homologous amplicons (same colour) with the same polarity are frequently recombining with a NAHR mechanism and that these deletions are the subsequent mutation events (Yen, 2001). Accordingly, the gr/gr deletions designated by Repping *et al.* (2003) are split into the three subgroups: g1/g2, r1/r3, r2/r4. Please note that the STS deletion pattern marking the different partial AZFb/c deletions are associated with the Y sequence of the R*-haplogroup and might be different in the Y chromosome of the other Y haplogroups. (C) Schematic overview on the putative 13 ampliconic AZFb/c inversion events in the Y–R* chromosome. To decrease the complexity of the picture the drawings for two further inversions (b1 ↔ b4; grey1 ↔ grey2) have been omitted. (D) Schematic view on the extensions of the so-called 'complete' or 'classical' AZFb and AZFc deletions and their overlap in the Y–R* chromosome. The two different breakage-fusion sites in the P5 palindromic (I and II) are indicated in the work of Repping *et al.* (2002).

Formal proof that the variable DAZ gene structures in exon 7 indeed form the molecular base for the polymorphic DYS1-49f blot fragments, the *TaqI* restriction patterns for each DAZ gene copy of the RP11-donor was extracted 'in silico' from the corresponding DAZ-BAC sequence data (Fernandes *et al.*, 2002; Jovelin *et al.*, 2003). The 10 kb long B was found in the DAZ1 and DAZ2 gene copy, the 7.15 kb long D2 and 3.1 kb long I fragments only in DAZ2, the F fragment (5 kb) and the A4 fragment (19.6 kb) in the DAZ3 gene and the A2 fragment (14.8 kb) only in the DAZ4 gene exon 7 repeat. No *TaqI* fragment with length of C (7.9 kb) could be identified in the DAZ locus of the RP-11 Y chromosome (Table II). According to the nomenclature of Torroni *et al.* (1990), the

corresponding DYS1 haplotype for the RP-11 donor should be then a variant of haplotype 'VIII' with the polymorphic *TaqI* fragments: A2, C0, D1, F1, and I1. This Y chromosomal haplotype has been reported with a median frequency in Caucasian male populations (5.6%) and absent in Africans (Torroni *et al.*, 1990). Although one origin of the polymorphic DYS1-*TaqI* fragments might be frequent single nucleotide mutations in DAZ exon 7 *TaqI* restriction sites (Jovelin *et al.*, 2003), additionally also real DAZ gene deletions and duplications should modify the *TaqI* restriction patterns of the DYS1-DAZ gene locus as well. They may include only the DAZ-repeat as described earlier (Vogt and Fernandes, 2003) or complete DAZ gene copies as described below.

Table II. DYS1-49f *TaqI* restriction fragments in *DAZ* locus of RP11 Y chromosome^a

DYS1 locus letter code	<i>DAZ1</i>	<i>DAZ2</i>	<i>DAZ3</i>	<i>DAZ4</i>	<i>in silico</i> 49f % homology
<i>A4</i>			19612		81
<i>A3</i>	– ^b	–	–	–	–
<i>A2</i>				14853	>80%
<i>A1</i>	–	–	–	–	81
<i>B</i>	10094	10100			>80%
<i>C</i>	–	–	–	–	–
<i>D1</i>		7152			81
<i>D2</i>	–	–	–	–	–
<i>E</i>	5854			5857 (2) ^c	97/83
<i>F1</i>			5008		83
<i>G</i>	4782	4781		4783	81
<i>H</i>	4020	4020		4021	83
<i>I</i>		3112			99/81
<i>J</i>	2975	2976	2975	2975	83
<i>K (DAZL)</i>	–	–	–	–	83
<i>L (DAZL)</i>	–	–	–	–	86
<i>M</i>	2076	2076	2075	2075	81
<i>N</i>	1992 (3)	1992	1992	1992 (3)	99/97
<i>O</i>	1877		1876	1876	99
<i>P</i>		1419 (3)		1419 (2)	80
<i>Q</i>	1237		1236	1236	81
<i>R</i>	966	966		966 (2)	80

^a All genomic fragments present in den RP-11 donors *DAZ*-BAC clone sequences and with a 'in silico' >80% homology to the 49f sequence (GenBank accession no.:AF414183) were listed and associated with the letter code of Ngo *et al.* (1996) in the first column (see also Figure 2). Italic letters mark the polymorphic fragments.

^b '–' indicate that these polymorphic fragment lengths are absent in the RP-11 *DAZ*-BAC sequences.

^c Numbers in parentheses indicate that this fragment length is found with 2, 3, ... copies.

A substantial number of additional polymorphic marker fragments in the *AZFb* and *AZFc* deletion intervals were found in recent years in different human populations (*AZFb*: LLY22g, 92R7, *AZFc*: poxY1), identified and studied first by blot experiments (Mathias *et al.*, 1994; Jobling *et al.*, 1996; Oakey and Tyler-Smith, 1990) then by the more convenient PCR format (Jobling and Tyler-Smith, 1995; Rosser *et al.*, 2000). One of the most popular Y population marker became the YAP element (Genome Data Base accession code: DYS287: 196899) with a unique origin and heterogenous frequency in different human populations (Hammer, 1995). YAP is the insertion of a short interspersed nucleotide element (SINE) *Alu-repeat* sequence in Yq11¹. Most of these highly repetitive sequence elements have an *AluI* restriction site in their 300 nucleotide long sequence unit and are therefore also called 'Alu-repeats' (Deininger *et al.*, 1981). The YAP insertion could be mapped to the proximal region of *AZFb* in the RP-11 BAC 169D1 sequence (GenBank accession no. AC010137) distal to the P4 palindrome (Figure 3A; S. Kirsch, personal commun.). YAP + chromosomes are frequently present in the Japanese (42%) and most other Asian populations although absent in the Taiwanese and with the highest frequency in the sub-Saharan African populations (Hammer and Horai, 1995).

Since all the described polymorphic sequence variants in the different *AZF* regions are broadly distributed in different human populations no reduced fertility is expected to be associated with any of them although there might be exceptions. A low fertility in men with haplogroup Y-hg26 + (now K* (xP), see below) was reported in a population of Denmark (Krausz *et al.*, 2001)

and in a Japanese men population the occurrence of azoospermia seems to be associated with distinct haplotypes of the polymorphic Yfm1 marker (Kuroki *et al.*, 1999; Ewis *et al.*, 2002) mapped distal to the *DAZ* gene copies in *AZFc* (Figure 3A).

Y chromosomal haplogroups are rooted in one complex pedigree

Since it was attractive to combine these highly informative sequence variants in 'compound' haplotypes structured in an hierarchical sequential order (Jobling and Tyler-Smith, 1995; Hurles and Jobling, 2001) more sequence variants of the Y chromosome were searched for extensively in the last years. However, only after it became possible to visualize sequence variants directly by specific heteroduplex formations in automated denaturing high performance liquid chromatography (DHPLC) experiments (Underhill *et al.*, 2000), the number of compound haplotypes became quickly expanding and the construction of a first comprehensive Y haplogroup pedigree became possible (Figure 4).

A detailed description of the 153 Y chromosomal haplogroups is beyond the scope of this review and the reader is advised to read the original papers (The Y Chromosome Consortium (YCC), 2002; Jobling and Tyler-Smith, 2003), respectively, to visit the corresponding websites (<http://ycc.biosci.arizona.edu>). The YCC nomenclature system marks the different Y chromosomal compound haplogroups (branches: A–R) in a unique hierarchical order. Analysis of a distinct marker set from this pedigree can be used to identify the haplogroup of each given

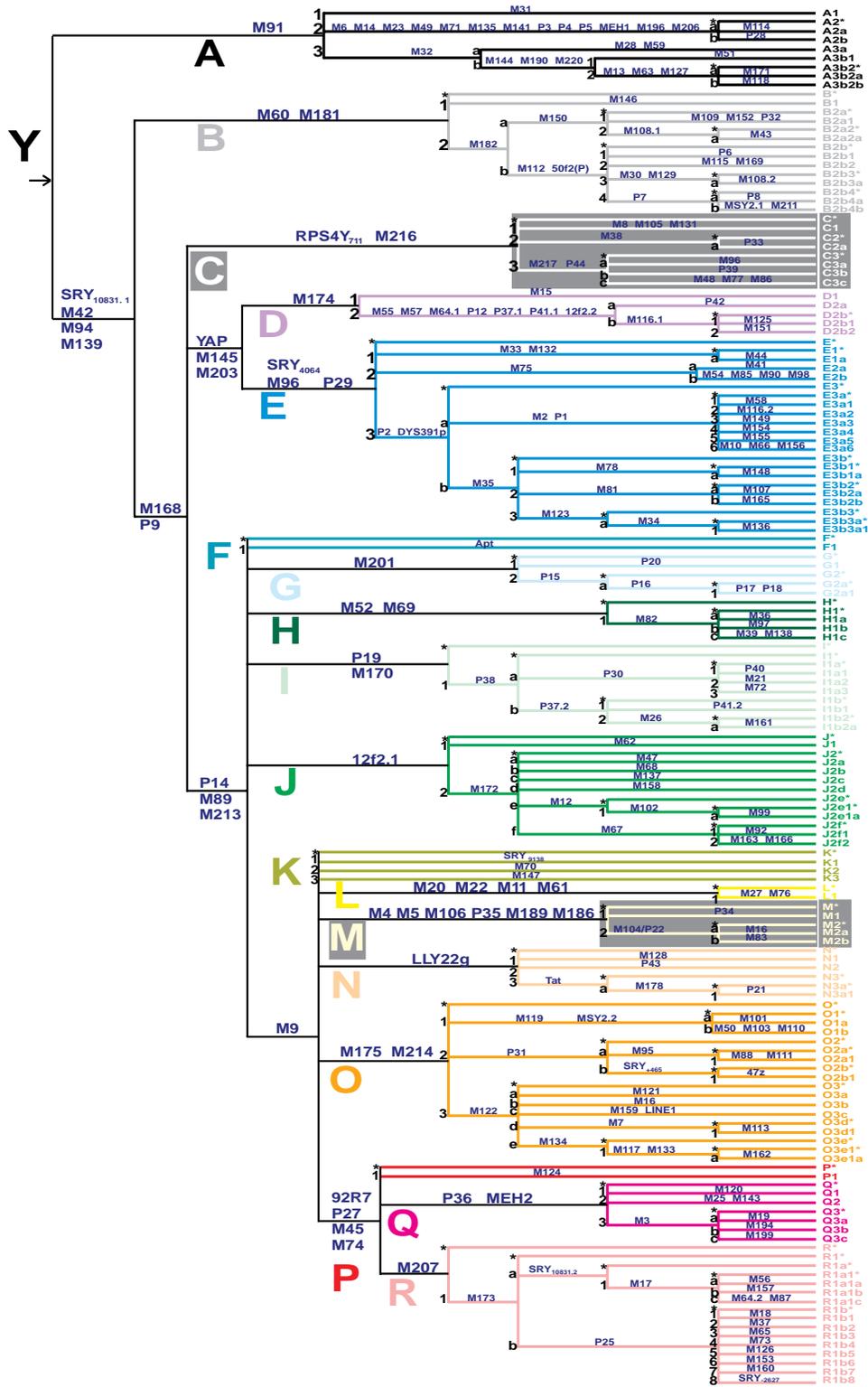


Figure 4. Schematic view of the phylogenetic tree of the 153 binary Y chromosomal haplogroups based on the references The Y chromosome Consortium (YCC) (2002) and Jobling and Tyler-Smith (2003) with modification of the colour code for better reading and distinguishing all Y haplogroups. The large letters A–R and Y at the left symbolise the main branches (clades) of the Y phylogeny. Along the horizontal pedigree-lines all bilallelic markers used for distinguishing the branches and subsequent haplogroups are given in blue colour. The nomenclature of the haplogroups for all branches is given at the right in the colour of the corresponding Y-branch. Haplogroups marked with an asterisk (R*) are ‘paragroups’ meaning that they are not further defined by a derived marker. More details of the markers, together with further information about the nomenclature rules can be found at the YCC website (<http://ycc.biosci.arizona.edu>).

Y DNA sequence (Paracchini *et al.*, 2002; Sanchez *et al.*, 2003; Brion *et al.*, 2005).

The published human Y DNA sequence belongs to Y chromosomal haplogroup R1*

The male specific region (MSY) of the human Y DNA sequence (35 Mb) has now been published (Skaletsky *et al.*, 2003) and deposited in different databases (GenBank: <http://www.ncbi.gov>; ENSEMBL: http://www.ensembl.org/Homo_sapiens/mapview?chr=Y). It is a mosaic of X-transposed, X-degenerated and repetitive Y-specific sequence blocks. The sequence is the result of a merged contig of bacterial artificial chromosome (BAC) clones each containing a portion of the MSY from the same individual, the RP-11 donor, an anonymous man of unknown fertility status. The only exception are the nine BAC clones spanning the *AZF_a* region. These were extracted earlier from two other BAC clone libraries derived from the CTA and CTB-coded male donor, respectively (Sun *et al.*, 1999). The Y short arm (Yp) contains a MSY sequence of approximately 8 Mb, the euchromatic Y long arm (Yq11) of approximately 14.5 Mb. The polymorphic heterochromatic Y region in the distal part of the Y long arm (Yq12) was estimated with ~40 Mb in the RP-11 donor encompassing at least three distinct sequence species, DYZ1, DYZ2, DYZ18 each of which forms long and homogenous tandem arrays (Skaletsky *et al.*, 2003). An interstitial heterochromatic repetitive sequence block of approximately 400 kb comprising >3000 tandem repeats of a 125 nt. sequence unit (DYZ19) interrupts the distal large X-degenerate sequence block in *AZF_b* (Figure 3).

No sequence informations were given for the centromeric repetitive alphoid DNA sequence block (~1 Mb). The pericentromeric region (~2 Mb) flanking this block in proximal Yq11 (Kirsch *et al.*, 2004) was also not included in this Y sequence. It was revealed only recently and is composed by segmental duplications of sequence blocks also located in the pericentromeric region of different autosomes, namely chromosomes 1, 2, 3, 19, 16, and 22 (Kirsch *et al.*, 2005). The pericentromeric Y chromosomal sequence region has therefore evolved more by *inter*-chromosomal than *intra*-chromosomal duplication events which have evolved the large repetitive sequence blocks ('amplicons') in distal Yq11 (Skaletsky *et al.*, 2003).

It has long been predicted that especially the Y long arm in Yq11 is composed of numerous Y-specific repetitive sequence blocks (Foote *et al.*, 1992; Kirsch *et al.*, 1996). Sequence analysis has now confirmed this assumption. However, beyond that it has revealed the unique pattern of large repetitive sequence blocks (amplicons) ranging in length between 115 kb and 678 kb in distal Yq11 (Figure 3). Most interesting, these amplicons were structurally assembled in palindrome structures in *AZF_b* and *AZF_c* (P1: 2.9 Mb; P2: 246 kb; P3: 736 kb; P4: 419 kb; P5: 996 kb) (Figure 3) and between the *AZF_a* and *AZF_b* deletion intervals (P6: 266 kb; P7: 30 kb; P8: 75 kb), respectively. Their arms 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 homologous palindrome arms revealed extensive homologies between 99.94–99.997% along the

complete amplicon sequence (Kuroda-Kawaguchi *et al.*, 2001; Repping *et al.*, 2002; Skaletsky *et al.*, 2003). This suggests that the functional integrity of the Y genes mapped in the ampliconic *AZF* sequence regions is maintained by frequent gene conversions—that is non-reciprocal transfer of sequence information—between the homologous palindrome arms (Rozen *et al.*, 2003).

The molecular extension of the three 'classical' *AZF* intervals in Yq11 is roughly ~8.73 Mb, that is ~60% of the complete Yq11 euchromatic sequence region. The *AZF_b* interval overlaps with the proximal part of the *AZF_c* deletion interval (Figure 3D) as was first proposed by a genomic YAC contig analysis (Kirsch *et al.*, 1996).

Considering the derived state of the marker M207 in position 139,206 in the RPCI-11 BAC clone 386L3 (GenBank accession no. AC006376) it can be deduced that the Y sequence derived from the RP-11 donor belongs to Y haplogroup R* (Figure 4). This finding could be confirmed and further specified to Y haplogroup R1* by a biallelic single nucleotide variant (SNV) marker in the yellow amplicon of the *AZF_c* region (Fernandes *et al.*, 2004).

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 would 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 must be concluded that in each of the 153 Y haplogroups identified (Figure 4) there should be a distinct Y reference sequence which is different from that of the RP-11 donor belonging to Y haplogroup R1*. Beyond of that the sequence variants in the highly polymorphic MSY1 locus and of the ~280 Y microsatellites (the so called Y STR-loci) are further specifying the Y sequences in each haplogroup establishing specific Y-lineages (Kayser *et al.*, 2000). These STR variants are also spread in the three *AZF* subintervals in Yq11 but not yet mapped precisely in comparison to the STS/SNV maps in the same intervals (Kayser *et al.*, 2004).

Mapping of AZF deletions in men of different Y haplogroups

The frequency of classical *AZF_a*, *AZF_b* and *AZF_c* deletions with a molecular extension first defined by molecular deletion mapping (Vogt *et al.*, 1996), then by the presence and absence of distinct border STS markers (*AZF_a*: Kamp *et al.*, 2001; *AZF_c*: Kuroda-Kawaguchi *et al.*, 2001; *AZF_b*: Repping *et al.*, 2002) seems not to be associated with a distinct Y chromosomal haplogroup (Paracchini *et al.*, 2000; Quintana-Murci *et al.*, 2001b; McElreavey and Quintana-Murci, 2003; Carvalho *et al.*, 2003, 2004), although the data of Blanco *et al.* (2000) and Paracchini *et al.* (2000) suggests that this might not be true for *AZF_a* deletions. Whether the different proximal border lines of the 'classical' *AZF_b* deletions (Repping *et al.*, 2002) are associated with some distinct Y haplogroups is not yet known. However, it can be safely assumed that the number of amplicons and palindromes in the structure of the *AZF_b* and *AZF_c* regions as shown in Figure 3 and associated with Y haplogroup R* is probably variable in at least some of the other Y haplogroups and an unknown number of

distinct ‘partial’ (to distinguish them from the molecular extensions of the classical complete *AZFb/c* deletions) *AZFb* and *AZFc* deletions would be the natural consequence (Yen, 2001; Hurler and Jobling, 2003). The NAHR based mechanism causing the intra-chromosomal recombinations and subsequent deletions in the ampliconic *AZF* sequence region is based on the same polarity of the homologous amplicons. The occurrence of b1/b3, g1/g2, r1/r3 and r2/r4 recombinations in the R*-Y-sequence resulting in distinct ‘partial’ *AZFb/c* deletions were therefore already predicted some years ago (Yen, 2001).

First partial *AZF* deletion studies pointed to a different number and structure of the *DAZ* genes in the red amplicons of the *AZFc* interval (Moro *et al.*, 2000; de Vries *et al.*, 2002; Fernandes *et al.*, 2002; Ferlin *et al.*, 2002, 2004; Ferras *et al.*, 2004). However, the analyses of the corresponding *AZFc* amplicon structures did not include an analysis of the associated Y chromosomal haplogroups and sometimes were restricted to only a qualitative *DAZ*-STS or -SNV deletion analysis. Considering the high rate of gene conversions in the same palindromic sequence regions (see previous chapter) the conclusion of an associated ‘real *DAZ* gene deletion’ drawn from solely a pattern of STS/SNV deletions was therefore probably sometimes wrong. Because of the inherent instability of the location of single nucleotide marker sites in the palindromic amplicons (Rozen *et al.*, 2003), the presence of a real partial *AZF* deletion needs to be generally confirmed by supporting experiments like FIBER-FISH (Repping *et al.*, 2003), or specific DNA blot experiments (Fernandes *et al.*, 2002), or similar quantitative gene copy deletion assays (Bienvenu *et al.*, 2001; Machev *et al.*, 2004). Additionally, if judged as being a clinically significant putative causative agent for the man’s infertility, the analysis of a DNA sample from the patient’s father (or brother) and identification of the family’s Y chromosomal haplogroup is strongly recommended.

Due to the generally high dynamic palindromic sequence structure in the ampliconic *AZFb* and *AZFc* sequence regions and the presence of similar albeit smaller repetitive sequence blocks along the whole sequence in Yq11 (Skaletsky *et al.*, 2003) multiple genomic rearrangements causing partial deletions in the ‘classical’ *AZF* sequence regions are expected. Most of them are expected to occur frequently in the different lineages of the human Y chromosome currently present in the global population of over three billion men. Methods which compare the age of the Y-lineage displaying a distinct variant of the R1*-*AZFb* and *AZFc* amplicon structure would then help to determine whether the structure identified is compatible with neutrality (i.e., fertility) or associated with some spermatogenic failure effects (Sabeti *et al.*, 2002). The preference of multi-copy genes especially in *AZFb* and *AZFc* can reflect genetic redundancy, but also some functional constraints from the germ line as for example representing a counterbalance for the unstable *AZFb/c* amplicon structure by reducing the risk of male infertility via a continuous genomic deletion/amplification mechanism in these *AZF* regions.

The first systematic screen for partial *AZF* deletions associated with specific Y chromosomal haplogroups was performed by Repping *et al.* (2003). In a study with some selected STS loci mapped in the *AZFb/c* amplicon structure of the R*-Y-sequence (Figure 3), 22 partial *AZFc* deletions were identified in a screen

of 689 individuals. These genomic deletions were confirmed by FIBER-FISH and summarized under the pseudonym ‘gr/gr’ deletions because they displayed the same deletion pattern of the green and red FISH signals in the men’s lymphocyte nuclei (Repping *et al.*, 2003). Another partial *AZF* deletion probably based on a b1/b3 recombination event in distal *AZFb* (Figure 3) was found in one individual. These FIBER-FISH confirmed partial *AZFb* and *AZFc* deletions were found in 9/246 men with a low sperm count ($<10 \times 10^6/\text{ml}$) compared with 0/148 men having a normal sperm count ($>40 \times 10^6/\text{ml}$), a difference with distinct statistical significance. It was therefore concluded that despite the frequent gr/gr deletions found also in men from other Y haplogroups, they must be associated with some aspects of male infertility, as is the case for the complete *AZFc* deletions, although of lower penetrance (Repping *et al.*, 2003).

However, the statistical significance of this conclusion would be lost if the 149th man with normal spermatogenesis in this study would be the first with a gr/gr FIBER-FISH pattern (9/246 versus 1/149, $P > 0.05$, Hurler and Tyler-Smith, 2005). This solely statistically based interpretation of the comparative experimental results must therefore be handled with caution. Moreover, assuming that the NAHR mechanism of all these recombinations needs a homologous sequence site as observed in autosomal NAHR events (Lupski, 1998), the collection of the large scale of possible recombination sites in the long green and red amplicons (~540 kb length) under the pseudonym ‘gr/gr’ recombinations is an unjustified oversimplification ignoring completely the high molecular complexity of these molecular events with ‘hot spots’ in distinct sequence regions (Machev *et al.*, 2004). The minimal segments for efficient processing (MEPS) homologous recombinations in mammalian meiosis are short duplicated sequence blocks of 132–232 identical nucleotides (Liskay and Stachelek, 1986). It can therefore be assumed that numerous NAHR sites with distinct recombination frequencies exist within the large g and r amplicons, respectively. They are not distinguished by the commonly used STS deletion assays but would need sequence analysis of the individual *AZFb/c*-breakage-fusion sequence as shown for the different *AZFa* deletions (Blanco *et al.*, 2000; Kamp *et al.*, 2000) and *AZFb* deletions (Repping *et al.*, 2002).

Consequently, the partial *AZF* deletions summarized under the pseudonym ‘gr/gr’ should be at least split in three different deletion types (1) those caused by recombinations between the g1/g2 amplicons, (2) between the r1/r3 amplicons, (3) between the r2/r4 amplicons (Figure 3B). It could be shown that gr/gr recombinations with subsequent partial *AZF* deletions have occurred independently in 14 different Y lineages (Repping *et al.*, 2003). Thus, a large variability of the exact NAHR sites in the g1/g2, r1/r3, and r2/r4 amplicons is expected but unknown until analysis of the specific breakage-fusion sequences.

The FIBER-FISH marked ‘gr/gr’ coined partial *AZF* deletions (FIBER-FISH experiments cannot distinguish between g1/g2, r1/r3 and r2/r4 based deletions) did not occur in each of the Y chromosomal haplogroups. Studying the *AZFb/c* amplicon structures of 368 men from different Y haplogroups no gr/gr deleted Y chromosomes were found in 29 branches whereas in 13 non-R1* branches only single gr/gr deletions were identified. Instead of this, in the 94 men from the R1* Y haplogroup 12 gr/gr deletions were reported (Repping *et al.*, 2003).

However, there is one significant exception. In the 12 men studied from the Y haplogroup D2b all were found with a *gr/gr* deleted Y chromosome (Repping *et al.*, 2003). This would account for an association of the *gr/gr* deleted Y chromosome with the D2b Y chromosome. Since the D2b haplogroup is common in Japan (~25%), it would suggest that the *gr/gr* deleted Y chromosomes are not impairing men's fertility at least in the Japanese men population.

Interestingly, in one man of Y lineage R1* and one of Q3 the FIBER-FISH experiments detected that after the proposed *gr/gr* deletion, subsequently an *AZF* duplication via the b2/b4 amplicons had occurred (Repping *et al.*, 2003). With reference to the *AZFc* gene content of the R1*-Y chromosome (Skaletsky *et al.*, 2003; Vogt, 2005), a b2/b4 duplication of a *gr/gr* deleted Y chromosome would restore the number of the *AZFc* gene copies and be a compensatory mutation event if the *gr/gr* deletion indeed would impair these men's fertility status. Unfortunately, the authors did not analyse the number of putative b2/b4 duplications in the Japanese men with a *gr/gr* deletion and the D2b haplogroup. Frequent subsequent b2/b4 duplication events would be expected if *gr/gr* deletions in this Y lineage would have an impact on the D2b men's fertility. Alternatively, men with a D2b Y chromosome are more likely to have some spermatogenic failure than those with another Y haplogroup (Repping *et al.*, 2003).

Although this study suggested that at least some partial *AZFc* deletions have some influence on the men's reproductive fitness, it is still preliminary since it did not distinguish the g and r amplicon based partial *AZFc* deletions. Moreover, their influence on the men's fertility is probably different in different Y lineages and its penetrance with respect to spermatogenic failure is certainly lower than for the complete *AZFc* deletions induced by b2/b4 recombinations. Whereas complete *AZFc* deletions were almost always found as 'de novo' deletions, i.e., only present in the patient's Y chromosome (Vogt, 1998; Krausz *et al.*, 2003), in all instances in which the father of an infertile *gr/gr* deleted man was available, the father's Y chromosome was also *gr/gr* deleted (Repping *et al.*, 2003; Machev *et al.*, 2004).

The first g1/g2 based recombination in *AZFb* leading to a partial deletion which included the *DAZI/DAZ2* gene doublet was reported by Fernandes *et al.* (2002). It was found as 'de novo' mutation event in five individuals with severe oligozoospermia and was confirmed with a specific DNA blot assay using the association of distinct *EcoRV* and *TaqI* fragments from the *DYS1* locus to specific *DAZ* gene copies of the Y chromosome from lineage R*. Since the same *DAZI/DAZ2* blot deletion pattern could not be identified in 107 fertile control samples, it was assumed that the g1/g2 deleted Y chromosomes are probably associated with spermatogenic failure. This conclusion has now been confirmed by three similar studies identifying a *DAZI/DAZ2* deletion in two men with incomplete meiotic arrest (Ferras *et al.*, 2004), in ten men with azoospermia or several grades of oligozoospermia (Ferlin *et al.*, 2005), and in four men with severe oligozoospermia (Giachini *et al.*, 2005). Most interestingly, the *DAZI/DAZ2* deletions found in the last study were always associated with deletion of the proximal *CDY1* gene copy (Giachini *et al.*, 2005) supporting the g1/g2 recombination mechanism as their putative origin.

Two further studies identified partial *AZFc* deletions in infertile men and fertile men (Hucklenbroich *et al.*, 2005; de Llanos *et al.*, 2005). Both were, however, based solely on PCR-assays using the

STS markers suggested by Repping *et al.* (2003) for the R1*-Y sequence: sY1258 proximal to b1, sY1161 distal to b1 and b2, sY1197 proximal to t2, sY1191 in u3, sY1291 distal in r2, sY1206 marking the ends of the g2 and g3 palindrome and sY1201 marking the distal border of the complete *AZFc* deletion interval (Figure 3). Accordingly, and only if the Y chromosome analysed has the same amplicon structure in distal Yq11 as known from the sequence of the R1*-Y chromosome and if no gene conversions have transferred their sites to other palindromic *AZF* sequence regions, b1/b3 deleted Y chromosomes should be marked by deletion of both sY1161 sites and deletion of sY1197, sY1191, sY1291 but presence of sY1258, sY1206 and sY1201 whereas the *gr/gr* deleted Y chromosomes are marked by deletion of the sY1291 site with presence of all other STSs. If, however, this is not the case as expected from at least some of the 153 other Y haplogroups (Fernandes *et al.*, 2004; Repping *et al.*, 2004; Machev *et al.*, 2004; see also Figure 3B and C) deletion of these markers might indicate some other still unknown Y chromosomal rearrangements.

In one screen genomic DNA samples of 283 individuals with some spermatogenic failure and of 232 fertile control samples were included (de Llanos *et al.*, 2005). It identified 12 putative *gr/gr* recombined Y chromosomes by sY1291 deletion in the infertile men population (i.e., 4.24%) and no sY1291 deletion in the Y chromosomes of the fertile men group. The highest frequency of sY1291 (*gr/gr*?) deleted Y chromosomes was found in the oligozoospermic men group (11/217). This distribution is opposite to that found for complete *AZFc* deletion with generally a higher incidence in the azoospermic men (Krausz *et al.*, 2003). The authors therefore also proposed that *gr/gr* deleted Y chromosomes might be not a cause for failure of spermatogenesis but only a distinct risk factor similar like mitochondrial DNA deletions (Kao *et al.*, 1998; Cummins, 2001). However, as stated above, since this study did not confirm the *gr/gr* association of their sY1291 deletions by appropriate FIBER-FISH or DNA blot experiments or other quantitative gene copy assays and also did not include a Y haplogroup analysis it cannot be excluded that the authors have identified some other Y chromosomal rearrangements in an *AZFb/c* amplicon structure which are different from that of the R*-Y-sequence and that the sY1291 deletion is not due to some *gr/gr* based real partial *AZF* deletion but due to a polymorphic STS deletion as recently found in men with Y haplogroup J (Machev *et al.*, 2004).

Interestingly, a second large (also solely PCR-based) STS screening study on *gr/gr* and additionally on b1/3 and b2/b3 deleted Y chromosomes, found no significant genotype/phenotype association (Hucklenbroich *et al.*, 2005). Screening 348 men with non-obstructive oligo/azoospermia and 170 men with normal spermatogenesis no impact of the identified sY1291-deleted Y chromosomes on the men's spermatogenesis profile could be noticed, the sperm count of men with the sY1291-deleted Y variant were all in the normal range. Moreover also b1/b3 and b2/b3 deleted Y chromosomes did not interfere with the fertility status of the men analysed. The authors identified also three novel partial *AZFc* deletions not fitting with their STS deletion pattern to a b1/b3; b2/b3 or *gr/gr* recombined *AZFc* amplicon structure of the R1*-Y chromosome. Unfortunately, no FISH or blot analyses were presented to confirm the novel *AZFc* deletions, nor did a Y haplogroup analysis reveal their possible association to a distinct Y lineage. Results of a Y haplogroup analysis

were only reported for the three fertile control samples with a sY1291-deleted Y chromosome and a normal sperm count (Hucklenbroich *et al.*, 2005). They belonged to Y haplogroup R1* (two individuals) and F*(one individual). Both Y lineages were most frequent in the population analysed and are known to contain frequently the gr/gr deleted AZFc sequence variant (Repping *et al.*, 2003).

A third large screening study for partial AZFc deletions was based not only on STS assays but also on the quantitative analysis of a number of gene markers for the DAZ and the CDY1 genes including some novel gene copy specific sequence variants. It also included FIBER-FISH experiments to distinguish between the presence of one or more DAZ gene doublets in the men's lymphocyte nuclei (Machev *et al.*, 2004) and distinguished the 153 Y haplogroups in the five major branches: Y(xD,E,J,P), DE, P; and J. Three different groups were screened for gr/gr deletions: (1) 300 infertile men with sperm counts between $0-131 \times 10^6/\text{ml}$, (2) 210 men of unknown fertility status and (3) 185 fertile men with one or more children. No significant difference was found in the frequency of gr/gr deleted Y chromosomes in the three groups analysed (Machev *et al.*, 2004).

However, considering the control groups with unknown sperm numbers (2 and 3) there might be a bias. If gr/gr deletions are indeed influencing the men's sperm numbers as originally stated (Repping *et al.*, 2003) the presence of oligozoospermic men must be excluded from these control groups. Only 'normospermic' men with normal fertility would be acceptable. A reduced sperm count does not necessarily cause infertility. Therefore, fertile men in the control groups with a gr/gr deletion in AZFc are expected to have a lower sperm count.

Indeed, when comparing the frequency of putative gr/gr-recombined AZFc structures in a group of normozoospermic ($n = 189$) and of oligo/azoospermic ($n = 150$) men the frequency of the gr/gr coined partial AZFc deletions were significantly higher in the oligo/azoospermic group (5.3%) than in the normozoospermic controls (0.5%) ($P < 0.012$) (Giachini *et al.*, 2005). This most recent study also confirmed that there is large heterogeneity in the partial 'gr/gr' coined AZF deletions distinguished by a marker of the CDY1 gene and an extreme heterogeneity of the phenotype ranging from azoospermia to normal sperm numbers. Also in the Machev-study (Machev *et al.*, 2004) overall 32 different gr/gr deletions were detected originating from at least 17 independent gr/gr recombination events.

Interestingly, in each Y haplogroup two gr/gr deleted Y chromosomes were identified with an associated b2 ↔ b3 or b3 ↔ b4 inversion (Machev *et al.*, 2004). It can therefore be predicted that the putative inversion events possible in the AZFb/c amplicon structure (Figure 3C) should occur with a similar frequency as the ampliconic recombination events associated with this Y haplogroup.

Mapping of AZF deletions in men with a Y(xR) AZF amplicon structure

The analysed marker deletion- and/or FIBER-FISH-patterns of some partial AZFc deletions could sometimes only be ordered along a continuous DNA segment if some inversions were allowed in the R*-AZFc amplicon structure (Machev *et al.*, 2004) leading to different polarities of the AZFc amplicons

(Figure 3C). The b2 ↔ b3 inversion with subsequent gr/gr recombination and associated AZFc deletion results in deletion of all markers of the AZFc-u3 single copy region including the polymorphic 50f2/C fragment. The b2 ↔ b3 inversion was first proposed to be present in the AZFb/c amplicon structure of men from a pre-N-haplogroup, because a g1/g3 recombination event leading to deletion of the DAZ3/DAZ4 gene doublet was found in all individuals from Y haplogroup N (Fernandes *et al.*, 2004). The g1/g3 deleted AZFc structure was marked by an extensive deletion pattern of STS/SNV markers analysing 37 positions along ~3000 kb of the R1*-AZFc amplicon structure and the DAZ3/DAZ4 deletion was confirmed by specific DNA blot analyses. A g1/g3 recombination is not expected in the R1*-AZFc amplicon structure because of the opposite polarity of g1 and g3 in this sequence (Figure 3A).

The same Y-N associated AZF amplicon structure was derived from a variant DAZ-FIBER-FISH pattern found in individuals with deletion of the sY1191 AZFc-u3 marker (Repping *et al.*, 2004). These authors concluded that not a b2 ↔ b3 inversion but a gr ↔ gr inversion ('gr-rg') in the R1*-AZFc amplicon structure with subsequent b2/b3 recombination would result in the Y-N AZFc amplicon structure. However, it must be admitted that both methods used for analysis of the different ampliconic rearrangements (DNA-blot and FIBER-FISH) are inadequate to distinguish both possibilities and that the variance of the FIBER-FISH patterns observed with deletion of the sY1191 marker can also point to different g1/g3 recombination sites in the pre-N*-AZFc amplicon structure.

The best guide to the proposed pre-N-AZFc amplicon structure may be probably provided by the Y chromosomes of the sister clade of the N-branch in the current Y phylogeny, namely haplogroup O (Figure 4). Here a b2 ↔ b3 inversion and not a gr ↔ gr inversion was accounted for the arrangement of the Y-O AZFc amplicon structure (Repping *et al.*, 2004). If this holds true, the Y-N deletion in AZFc would be the result of a g1/g3 recombination based on a pre-N b2 ↔ b3 inversion, rather than of a b2/b3 recombination. The same conclusion was also drawn from the studies of Machev and coworkers and extends to the observation that the same b2 ↔ b3 rearrangement might be not only present in men of Y-N but also in men of Y-F* and Y-I (Machev *et al.*, 2004). This was also found in a study of 1563 individuals by Repping *et al.* (2004).

It has been already appreciated some years ago that just the sequence area of the u3 AZFc region is particularly vulnerable for genomic rearrangements. At least six independent deletions and four duplications affect a short section of this sequence region which is marked by the polymorphic DYS7-50f2/C fragment (Figure 3A). Together these u3 linked AZFb/c rearrangements are present in ~8% of normal men (Jobling *et al.*, 1996). The u3-marker sY1192 is only ~13.5 kb proximal to the 50f2/C u3-AZFc section (Fernandes *et al.*, 2004). Considering the fact that we know the origin of probably only three of the proposed six 50f2/C deletions now and no origins for the 50f2/C duplication events we must conclude that the molecular base of most of the ten proposed rearrangements in the AZFc-u3 sequence block, including the 50f2/C site, are still unknown.

Based on these earlier analyses and the now known R1*-Y chromosome associated AZFb/c amplicon structure it can be predicted that more rearrangements than described above will exist

in at least some of the non-R*-Y haplogroups and that these will lead to more partial *AZFb* and *AZFc* deletions not yet identified. Their detection might probably need more sophisticated methods than FIBER-FISH or genomic DNA restriction assays due to the high frequencies of sequence conversions observed in the palindromic organisation of the *AZFc* amplicon structure (Rozen *et al.* 2003). To be on the safe side, it seems therefore wise not to restrict any partial *AZF* deletion analysis to a simple PCR format if one wants to draw some conclusions from the identified *AZF*-STS deletions to its putative association with the patient's testicular pathology.

Are some *AZFa* and *AZFb* deletions associated with distinct Y haplogroups ?

The 'classical' *AZFa* deletions caused after HERV15yq1/y2 recombination events in proximal Yq11 and including both *AZFa* genes, *USP9Y* and *DBY*, are expected to occur with a different frequency in Y chromosomal haplogroups with and without the L1tr element in the distal HERV15yq2 sequence block (Blanco *et al.*, 2000). The deletion of the 12f2-2 kb sequence of the *DYS11* locus has occurred independently in at least two different Y lineages. It cuts out precisely the *L1PA4* sequence, a truncated LINE element (Kamp *et al.*, 2000). This would result in a substantially longer segment of sequence identity between both HERV15 elements. An increased rate of HERV15yq1/yq2 recombinations resulting subsequently in the classical *AZFa* deletions would be therefore expected in men with a Y chromosome of the 12f2-2 kb haplogroups (Blanco *et al.*, 2000).

Partial *AZFa* deletions including only the *USP9Y* gene (Qureshi *et al.*, 1996; Blagosklonova *et al.*, 2000) or *DBY* gene (Foresta *et al.*, 2000; van Landuyt *et al.*, 2001) were repeatedly reported but without any analysis of the associated Y haplogroups. Since the complete Yq11 sequence is structured with multiple duplicated sequence blocks shorter than the prominent *AZFb/c* amplicons but long enough for multiple homologous NAHR based recombinations (Liskay and Stachelek, 1986), it is most likely that these partial *AZFa* deletions are due to some still unknown NAHR events in the corresponding sequence areas and it would be interesting to learn whether they are associated with one or more distinct Y lineages. This linkage is suggested because the occurrence of partial *AZFa* deletions seemed to be restricted to distinct populations, they were not found in any large screening surveys for *AZF* deletions (Vogt, 1998; Simoni, 2001, Krausz *et al.*, 2003).

Considering the polymorphic deletion of the 50f2/E sequence together with the 50f2/C sequence and the *RBMV* gene copies (Jobling *et al.*, 1996) and presence of the polymorphic LLY22g marker in distal *AZFb* (Kirsch *et al.*, 1996) a polymorphic structure in the unique distal *AZFb* sequence, i.e., not overlapping with the *AZFc* deletion interval, is expected. The variant and polymorphic exon structure of the *RBMV* gene copies in the same Y region (Prosser *et al.*, 1996, Yen, 1999) and the identification of some unique partial *AZFb* deletions identified in four infertile Italian men (Ferlin *et al.* 2003) seem to support this conclusion. The Italian partial *AZFb* deletion can not be explained by any ampliconic recombination event from the R1*-*AZFb/c* amplicon structure. An intriguing possibility might

therefore be the presence of some chromosomal rearrangements in the distal *AZFb* sequence associated with the specific Y lineage of these patient's families. Most interesting, these partial *AZFb* deletions are probably not a polymorphic neutral deletion event since they were confirmed as a 'de novo' mutation in two individuals with distinct testicular pathologies (Ferlin *et al.*, 2003).

Are *AZF* deletions and duplications two sides of the same NAHR medal ?

If NAHR is the molecular mechanism causing subsequently the observed complete and partial *AZFa*, *AZFb* and *AZFc* deletions, it can be predicted that the reciprocal events, *AZFa*, *AZFb* and *AZFc* duplications, will be also generated (Potocki *et al.*, 2000). Molecular duplications are probably rarely pathogenic and harder to detect because they require a quantitative rather than qualitative assay. Cytogenetically visible duplications of the whole Y chromosome (46,XY) usually results in male infertility because of mistakes in the meiotic segregation of the sex chromosomes in the nuclei of the patient's spermatocytes (Solari and Rey Valzacchi, 1997; Rives *et al.*, 2003). Duplications of part of the Yq11 euchromatin forming the dicentric dic-(Yp) or Ynf chromosome are always associated with male infertility (see previous chapter).

Surprisingly, in contrast of this, duplication of the *DAZ*-*AZFc* interval and translocation to the proximal part of the Y short arm seems to be compatible with human fertility (Engelen *et al.*, 2003). This Y chromosomal rearrangement reminds to the occurrence of a similar inversion event in the Y chromosome of the Gujerati Muslim Indian population in South Africa (Bernstein *et al.*, 1986). Although there are not yet any molecular experiments supporting this view, it can be assumed that both Y rearrangements are probably based on the similar sequence structure of proximal Yp and the distal *AZFb* region in Yq11. Looking at the R1*-Y sequence structure (Skaletsky *et al.*, 2003), copies and pseudo-gene copies of the *RBMV*, *TSPY*, and *PRY* genes were mapped to both these Y regions.

Submicroscopically smaller (molecular) *AZF* duplications can be estimated with different molecular methods: (1) by a semi-quantitative analysis of STR markers mapped inside and outside the duplicated *AZF* region comparing their peak densities in denaturing sequence gels, (2) by a quantitative DNA blot experiment with probes hybridising inside and outside the duplicated *AZF* region comparing their autoradiographic signal intensities, (3) with FIBER-FISH experiments on the duplicated *AZF* chromatin domain with Y-specific cosmid clones.

Two duplications of the *AZFa* region mediated by the HERV15 sequence blocks in proximal Yq11 have been revealed during a population survey of 9 Y-STRs mapped in the *AZFa* region (Bosch and Jobling, 2003). Both duplications seemed not to interfere with the men's fertility status. They might therefore be widespread and present in different Y haplogroups, similar as found earlier for the 50f2/C site in proximal *AZFc* (Jobling *et al.*, 1996). One of the 50f2/C marked duplications in proximal *AZFc* might be the origin of the seven *DAZ* gene copies observed earlier in some fertile individuals by FIBER-FISH (Glaeser *et al.*, 1998). Duplications in *AZFc* followed after a gr/gr based partial *AZFc* deletion are the b2/b4 duplications also identified by

FIBER-FISH (Repping *et al.*, 2003). It can be concluded that similar polymorphic duplications are present along the whole Yq11 sequence region due to its high amount of repetitive sequence blocks. They might be probably triggered by continuous genomic deletion/amplification mechanisms selected in the different Y haplogroups for balancing their male's fertility status.

Summary

Obviously we are just beginning to understand the dynamic structure of the human Y chromosome and the putative range of its possible rearrangements. All the observed molecular variations are probably based primarily on its extraordinary repetitive sequence structure. This is especially true for the Yq11 euchromatic sequence region where the *AZF* locus resides embedded in different polymorphic intervals historically divided in *AZF*a, *AZF*b and *AZF*c (Vogt *et al.*, 1996). Quantitative blot analysis (Kirsch *et al.*, 1996) and then sequence analysis (Skaletsky *et al.*, 2003) has shown that there is a large overlap between the *AZF*b and *AZF*c deletion intervals and that most Y genes expressed solely in human testes tissue are deleted with the *AZF*b deletion (Vogt, 2005).

Considering the variable copy number of these Y genes in Y haplogroup D2b, F(xH,K), I and N and probably more Y haplogroups not yet identified, the question is raised which Y genes in these polymorphic *AZF*b/c subintervals are really essential for spermatogenesis, that means which gene deletion is really a causative agent for the clinically observed man's testicular pathology and which gene deletion is neutral (polymorphic) because the gene is only balancing and shaping the reproductive fitness factor(s) of the male in the different human populations and if deleted is counterbalanced by another beneficial Y or non-Y male fertility factor still unknown (see also Quintana-Murci *et al.*, 2001a; Vogt, 2004).

In this context it is worth to consider also the choice of the Y reference sequence, which was in fact accidentally extracted from a man with an unknown fertility status now known to belong to Y lineage R* (Fernandes *et al.*, 2004). If this Y sequence would have been derived from a man from Y lineage N*, our view on the *AZF*c amplicon structure would have been simpler but we would have also no sequence data from the *AZF*c-u3 segment. Consequently, the R*-Y sequence now available may also lack sequence regions which are present on the Y chromosome from another Y haplogroup.

In many cases there are alternative recombinatorial routes for the homologous amplicons leading to the same *AZF*b/c amplicon structure as discussed here for the *AZF*c structure of Y lineage N*. If derived from the direct polarity pattern of the R*-*AZF*c amplicon blocks, a b2/b3 inversion followed by a g1/g3 recombination in the pre-N* lineage is most likely (Fernandes *et al.*, 2004; Machev *et al.*, 2004) but another route based on a gr/gr inversion (Repping *et al.*, 2004) or the route based on an inversion of the yellow amplicons (P1.1/P1.2) (Machev *et al.*, 2004) can not be excluded. STS deletion analysis but also FIBER-FISH and blot analysis are probably not suitable to identify and to distinguish these possibilities. Additional analyses of sequences and structural maps from the Y chromosome of other lineages –perhaps choosing first the most divergent

A haplogroup (The Y Chromosome Consortium (YCC), 2002)– are therefore highly desirable.

Acknowledgements

I like to thank Profs Ann Chandley (University of Edinburgh), Erika Bühler (University of Basel) and Richard Pfeiffer (University of Erlangen) for having introduced me in the complexity of Y chromosomal rearrangements. I am indebted to Chris Tyler-Smith for paving me the way towards a molecular understanding of the complexity of the Y chromosomal haplogroups in different human populations. My students Susana Fernandes, Christine Kamp, Stefan Kirsch, and Mike Koehler and my technicians, Angela Edelmann and Karin Hüllen are thanked for their valuable experimental contributions to the analysis of the distinct Y chromosomal rearrangements in infertile men and fertile men and the associated variabilities of the *AZF* genes. Mark Jobling is thanked for sharing with me a novel edition of the Y haplogroup pedigree presented in Figure 4. Prof Dr Thomas Strowitzki is thanked for his continuous clinical support of the *AZF* gene project and Mrs. Christine Mahrla and Uli Mueller is thanked for their extensive contributions to the final version of this manuscript.

References

- Affara NA, Florentin L, Morrison N, Kwok K, Mitchell M, Cook A, Jamieson D, Glasgow L, Meredith L, Boyd E et al. (1986) Regional assignment of Y-linked DNA probes by deletion mapping and their homology with X-chromosome and autosomal sequences. *Nucleic Acids Res* 14,5353–5373.
- Andersson M, Page DC, Pettay D, Subrt I, Turleau C, de Grouchy J and de la Chapelle A (1988) Y;autosome translocations and mosaicism in the aetiology of 45,X maleness: assignment of fertility factor to distal Yq11. *Hum Genet* 7,2–7.
- Armstrong SJ, Kirkham AJ and Hulten MA (1994) XY chromosome behaviour in the germ-line of the human male: a FISH analysis of spatial orientation, chromatin condensation and pairing. *Chromosome Res* 2,445–452.
- Ashley T (1984) A re-examination of the case for homology between the X and Y chromosomes of mouse and man. *Hum Genet* 67,372–377.
- Baarends WM, Wassenaar E, van der Laan R, Hoogerbrugge J, Sladdens-Linkels E, Hoeijmakers JHJ, de Boer P and Grootegoed JA (2005) Silencing of unpaired chromatin and histone H2A ubiquitination in mammalian meiosis. *Mol Cell Biol* 25,1041–1053.
- Bardoni B, Zuffardi O, Guioli S, Ballabio A, Simi P, Cavalli P, Grimoldi MG, Fraccaro M and Camerino G (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.
- Bernstein R, Wade A, Rosendorff J, Wessels A and Jenkins T (1986) Inverted Y chromosome polymorphism in the Gujerati Muslim Indian population of South Africa. *Hum Genet* 74,223–239.
- Bienvenu T, Patrat C, McElreavey K, de Almeida M and Jouannet P (2001) Reduction in the DAZ gene copy number in two infertile men with impaired spermatogenesis. *Ann Genet* 44,125–128.
- Bishop C, Guellaen G, Geldwerth D, Fellous M and Weissenbach J (1984) Extensive sequence homologies between Y and other human chromosomes. *J Mol Biol* 173,403–417.
- Blagosklonova O, Fellmann F, Clavequin MC, Roux C and Bresson JL (2000) *AZF*a deletions in Sertoli-cell-only syndrome: a retrospective study. *Mol Hum Reprod* 6,795–799.
- Blanco P, Shlumukova M, Sargent CA, Jobling MA, Affara N and Hurler ME (2000) Divergent outcomes of intrachromosomal recombination on the human Y chromosome: male infertility and recurrent polymorphism. *J Med Genet* 37,752–758.
- Bosch E and Jobling MA (2003) Duplications of the *AZF*a region of the human Y chromosome are mediated by homologous recombination between HERVs and are compatible with male fertility. *Hum Mol Genet* 12,341–347.
- Brion M, Sobrino B, Blanco-Verea A, Lareu MV and Carracedo A (2005) Hierarchical analysis of 30 Y chromosome SNPs in European populations. *Int J Legal Med* 119,10–15.

- Carvalho CM, Fujisawa M, Shirakawa T, Gotoh A, Kamidono S, Freitas Paulo T, Santos SE, Rocha J, Pena SD and Santos FR (2003) Lack of association between Y chromosome haplogroups and male infertility in Japanese men. *Am J Med Genet A* 116,152–158.
- Carvalho CM, Rocha JL, Santos FR, Kleiman SE, Paz G, Yavetz H and Pena SD (2004) Y chromosome haplotypes in azoospermic Israeli men. *Hum Biol* 76,469–478.
- Casanova M, Leroy P, Boucekkinne C, Weissenbach J, Bishop C, Fellous M, Purrello M, Fiori G and Siniscalco M (1985) A human Y-linked DNA polymorphism and its potential for estimating genetic and evolutionary distance. *Science* 230,1403–1406.
- Chandley AC, Goetz P, Hargreave TB, Joseph AM and Speed RM (1984) On the nature and extent of XY pairing at meiotic prophase in man. *Cytogenet Cell Genet* 38,241–247.
- Chandley AC, Ambros P, McBeath S, Hargreave TB, Kilanowski F and Spowart G (1986) Short arm dicentric Y chromosome with associated statural defects in a sterile man. *Hum Genet* 73,350–353.
- Charlesworth B (2003) The organization and evolution of the human Y chromosome. *Genome Biol* 4,226.
- Cummins J (2001) Mitochondrial DNA and the Y chromosome: parallels and paradoxes. *Reprod Fertil Dev* 13,533–542.
- Deininger PL, Jolly DJ, Rubin CM, Friedmann T and Schmid CW (1981) Base sequence studies of 300 nucleotide renatured repeated human DNA clones. *J Mol Biol* 151,17–33.
- Diekmann L, Palm K, Pfeiffer RA, Trautmann U, Scholz W, Schroers E, Vogt P and Köhler M (1992) Multiple minute marker chromosomes derived from Y identified by FISH in an intersexual infant. *Hum Genet* 90,181–183.
- Disteche CM, Casanova M, Saal H, Friedman C, Sybert V, Graham J, Thuline H, Page DC and Fellous M (1986) Small deletions of the short arm of the Y chromosome in 46,XY females. *Proc Natl Acad Sci USA* 83,7841–7844.
- Engelen JJ, Arens YH, Gondrie ET, Alofs MG, Loneus WH and Hamers AJ (2003) Intrachromosomal insertion translocation resulting in duplication of chromosome band Yq11.2 in two fertile brothers. *Am J Med Genet A* 118,287–289.
- Ewis AA, Lee J, Shinka T and Nakahori Y (2002) Microdeletions of a Y-specific marker, Yfm1, and implications for a role in spermatogenesis. *J Hum Genet* 47,257–261.
- Faed MJ, Lamont MA and Baxby K (1982) Cytogenetic and histological studies of testicular biopsies from subfertile men with chromosome anomaly. *J Med Genet* 19,49–56.
- Ferlin A, Moro E, Rossi A and Foresta C (2002) A novel approach for the analysis of DAZ gene copy number in severely idiopathic infertile men. *J Endocrinol Invest* 25,RC1–RC3.
- Ferlin A, Moro E, Rossi A, Dallapiccola B and Foresta C (2003) The human Y chromosome's azoospermia factor b (AZFb) region: sequence, structure, and deletion analysis in infertile men. *J Med Genet* 40,18–24.
- Ferlin A, Bettella A, Tessari A, Salata E, Dallapiccola B and Foresta C (2004) Analysis of the DAZ gene family in cryptorchidism and idiopathic male infertility. *Fertil Steril* 81,1013–1018.
- Ferlin A, Tessari A, Ganz F, Marchina E, Barlati S, Garolla A, Engl B and Foresta C (2005) Association of partial AZFc region deletions with spermatogenic impairment and male infertility. *J Med Genet* 42,209–215.
- Fernandes S, Huellen K, Goncalves J, Dukal H, Zeisler J, Rajpert De Meyts E, Skakkebaek NE, Habermann B, Krause W, Sousa M et al. (2002) High frequency of DAZ1/DAZ2 gene deletions in patients with severe oligozoospermia. *Mol Hum Reprod* 8,286–298.
- Fernandes S, Paracchini S, Meyer LH, Florida G, Tyler-Smith C and Vogt PH (2004) A large AZFc deletion removes DAZ3/DAZ4 and nearby genes from men in Y haplogroup N. *Am J Hum Genet* 74,180–187.
- Ferras C, Fernandes S, Marques CJ, Carvalho F, Alves C, Silva J, Sousa M and Barros A (2004) AZF and DAZ gene copy-specific deletion analysis in maturation arrest and Sertoli cell-only syndrome. *Mol Hum Reprod* 10,755–761.
- Footo S, Vollrath D, Hilton A and Page DC (1992) The human Y chromosome: overlapping DNA clones spanning the euchromatic region. *Science* 258,60–66.
- Foresta C, Ferlin A and Moro E (2000) Deletion and expression analysis of AZFa genes on the human Y chromosome revealed a major role for DBY in male infertility. *Hum Mol Genet* 9,1161–1169.
- Fryns JP, Cassiman JJ and Van den Berghe H (1978) Unusual in vivo rearrangements of the Y chromosome with mitotic instability in vitro. *Hum Genet* 44,349–355.
- Giachini C, Guarducci E, Longepied G, Degl'Innocenti S, Becherini L, Forti G, Mitchell MJ and Krausz C (2005) The gr/gr deletion(s): a new genetic test in male infertility? *J Med Genet Epub doi: 10.1136 in press.*
- Gill TJ (2002) Evolutionary genetics and infertility. *Am J Reprod Immunol* 48,43–49.
- Glaeser B, Yen PH and Schempp W (1998) Fibre-fluorescence in situ hybridization unravels apparently seven DAZ genes or pseudogenes clustered within a Y chromosome region frequently deleted in azoospermic males. *Chromosome Res* 6,48148–48146.
- Hammer MF (1995) A recent common ancestry for human Y chromosomes. *Nature* 378,376–380.
- Hammer MF and Horai S (1995) Y chromosomal DNA variation and the peopling of Japan. *Am J Hum Genet* 56,951–962.
- Hartung M, Devictor M, Codaccioni JL and Stahl A (1988) Yq deletion and failure of spermatogenesis. *Ann Genet* 31,21–26.
- Henegariu O, Pescovitz OH, Vance GH, Verbrugge J and Heerema NA (1997) A case with mosaic di-, tetra-, and octacentric ring Y chromosomes. *Am J Med Genet* 71,426–429.
- Hucklenbroich K, Gromoll J, Heinrich M, Hohoff C, Nieschlag E and Simoni M (2005) Partial deletions in the AZFc region of the Y chromosome occur in men with impaired as well as normal spermatogenesis. *Hum Reprod* 20,191–197.
- Hurles ME and Jobling MA (2001) Haploid chromosomes in molecular ecology: lessons from the human Y. *Mol Ecol* 10,1599–1613.
- Hurles ME and Jobling MA (2003) A singular chromosome. *Nat Genet* 34,246–247.
- Hurles ME and Tyler-Smith C (2005) Genomic disorders: the genomic basis of disease. In Lupski JR and Stankiewicz PT (eds) *Humana Press Inc, Totowa, New Jersey, USA, in press.*
- Jaruzelska J, Korcz A, Wojda A, Jedrzejczak P, Bierla J, Surmacz T, Pawelczyk L, Page DC and Kotecki M (2001) Mosaicism for 45,X cell line may accentuate the severity of spermatogenic defects in men with AZFc deletion. *J Med Genet* 38,798–802.
- Jobling MA and Tyler-Smith C (1995) Fathers and sons: the Y chromosome and human evolution. *Trends Genet* 11,449–456.
- Jobling MA and Tyler-Smith C (2003) The human Y chromosome: an evolutionary marker comes of age. *Nat Rev Genet* 4,598–612.
- Jobling MA, Samara V, Pandya A, Fretwell N, Bernasconi B, Mitchell RJ, Gerelsaikhan T, Dashnyam B, Sajantila A, Salo PJ et al. (1996) Recurrent duplication and deletion polymorphisms on the long arm of the Y chromosome in normal males. *Hum Mol Genet* 5,1767–1775.
- Jobling MA, Bouzekri N and Taylor PG (1998) Hypervariable digital DNA codes for human paternal lineages: MVR-PCR at the Y-specific minisatellite, MSY1 (DYF155S1). *Hum Mol Genet* 7,643–653.
- Jobling MA, Heyer E, Dieltjes P and de Knijff P (1999) Y chromosome-specific microsatellite mutation rates re-examined using a minisatellite, MSY1. *Hum Mol Genet* 8,2117–2120.
- Jovelin F, Berthaud S and Lucotte G (2003) Molecular basis of the TaqI p49a,f polymorphism in the DYS1 locus containing DAZ genes. *Mol Hum Reprod* 9,509–516.
- Kamp C, Hirschmann P, Voss H, Huellen K and Vogt PH (2000) Two long homologous retroviral sequence blocks in proximal Yq11 cause AZFa microdeletions as a result of intrachromosomal recombination events. *Hum Mol Genet* 9,2563–2572.
- Kamp C, Huellen K, Fernandes S, Sousa M, Schlegel PN, Mielnik A, Kleiman S, Yavetz H, Krause W, Kupker W et al. (2001) High deletion frequency of the complete AZFa sequence in men with Sertoli-cell-only syndrome. *Mol Hum Reprod* 7,987–994.
- Kao SH, Chao HT and Wei YH (1998) Multiple deletions of mitochondrial DNA are associated with the decline of motility and fertility of human spermatozoa. *Mol Hum Reprod* 4,657–666.
- Kayser M, Roewer L, Hedman M, Henke L, Henke J, Brauer S, Kruger C, Krawczak M, Nagy M, Dobosz T et al. (2000) Characteristics and frequency of germline mutations at microsatellite loci from the human Y chromosome, as revealed by direct observation in father/son pairs. *Am J Hum Genet* 66,1580–1588.
- Kayser M, Kittler R, Erler A, Hedman M, Lee AC, Mohyuddin A, Mehdi SQ, Rosser Z, Stoneking M, Jobling MA et al. (2004) A comprehensive survey of human Y chromosomal microsatellites. *Am J Hum Genet* 74,1183–1197.
- Kirsch S, Keil R, Edelmann A, Henegariu O, Hirschmann P, LePaslier D and Vogt PH (1996) Molecular analysis of the genomic structure of the human Y chromosome in the euchromatic part of its long arm (Yq11). *Cytogenet Cell Genet* 75,197–206.

- Kirsch S, Weiss B, Zumbach K and Rappold G (2004) Molecular and evolutionary analysis of the growth-controlling region on the human Y chromosome. *Hum Genet* 114,173–181.
- Kirsch S, Weiss B, Miner TL, Waterston RH, Clark RA, Eichler EE, Munch C, Schempp W and Rappold G (2005) Interchromosomal segmental duplications of the pericentromeric region on the human Y chromosome. *Genome Res* 15,195–204.
- Köhler MR and Vogt PH (1994) Interstitial deletions of repetitive DNA blocks in dicentric human Y chromosomes. *Chromosoma* 103,324–330.
- Krausz C, Quintana-Murci L and McElreavey K (2000) Prognostic value of Y deletion analysis: what is the clinical prognostic value of Y chromosome microdeletion analysis? *Hum Reprod* 15,1431–1434.
- Krausz C, Quintana-Murci L, Rajpert-De Meyts E, Jorgensen N, Jobling MA, Rosser ZH, Skakkebaek NE and McElreavey K (2001) Identification of a Y chromosome haplogroup associated with reduced sperm counts. *Hum Mol Genet* 10,1873–1877.
- Krausz C, Forti G and McElreavey K (2003) The Y chromosome and male fertility and infertility. *Int J Androl* 26,70–75.
- Kuroda-Kawaguchi T, Skaletsky H, Brown LG, Minx PJ, Cordum HS, Waterston RH, Wilson RK, Silber S, Oates R, Rozen S et al. (2001) The AZFc region of the Y chromosome features massive palindromes and uniform recurrent deletions in infertile men. *Nat Genet* 29,279–286.
- Kuroki Y, Iwamoto T, Lee J, Yoshiike M, Nozawa S, Nishida T, Ewis AA, Nakamura H, Toda T, Tokunaga K et al. (1999) Spermatogenic ability is different among males in different Y chromosome lineage. *J Hum Genet* 44,289–292.
- Landuyt van L, Lissens W, Stouffs K, Tournaye H, Van Steirteghem A, Liebaers I, Blagosklonova O and Bresson JL (2001) The role of USP9Y and DBY in infertile patients with severely impaired spermatogenesis. *Mol Hum Reprod* 7,691–693.
- Liskay RM and Stachelek JL (1986) Information transfer between duplicated chromosomal sequences in mammalian cells involves contiguous regions of DNA. *Proc Natl Acad Sci USA* 83,1802–1806.
- de Llanos M, Luis Balleca J, Gazquez C, Margarit E and Oliva R (2005) High frequency of gr/gr chromosome Y deletions in consecutive oligospermic ICSI candidates. *Hum Reprod* 20,216–220.
- Lucotte G and Ngo NY (1985) p49F, A highly polymorphic probe, that detects TaqI RFLPs on the human Y chromosome. *Nucleic Acids Res* 13,8285.
- Lupski JR (1998) Genomic disorders: structural features of the genome can lead to DNA rearrangements and human disease traits. *Trends Genet* 14,417–422.
- Ma K, Sharkey A, Kirsch S, Vogt P, Keil R, Hargreave TB, McBeath S and Chandley AC (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. *Hum Mol Genet* 1, 29–33.
- Ma K, Inglis JD, Sharkey A, Bickmore WA, Hill RE, Prosser EJ, Speed RM, Thomson EJ, Jobling M, Taylor K et al. (1993) A Y chromosome gene family with RNA-binding protein homology: candidates for the azoospermia factor AZF controlling human spermatogenesis. *Cell* 75, 1287–1295.
- Machev N, Saut N, Longepied G, Terriou P, Navarro A, Levy N, Guichaoua M, Metzler-Guillemain C, Collignon P, Frances AM et al. (2004) Sequence family variant loss from the AZFc interval of the human Y chromosome, but not gene copy loss, is strongly associated with male infertility. *J Med Genet* 41,814–825.
- Mathias N, Bayes M and Tyler-Smith C (1994) Highly informative compound haplotypes for the human Y chromosome. *Hum Mol Genet* 3,115–123.
- McElreavey K and Quintana-Murci L (2003) Male reproductive function and the human Y chromosome: is selection acting on the Y? *Reprod Biomed Online* 7,17–23.
- Moro E, Ferlin A, Yen PH, Franchi PG, Palka G and Foresta C (2000) Male infertility caused by a de novo partial deletion of the DAZ cluster on the Y chromosome. *J Clin Endocrinol Metab* 85,4069–4073.
- Ngo KY, Vergnaud G, Johnsson C, Lucotte G and Weissenbach J (1986) A DNA probe detecting multiple haplotypes of the human Y chromosome. *Am J Hum Genet* 38,407–418.
- Oahey R and Tyler-Smith C (1990) Y chromosome DNA haplotyping suggests that most European and Asian men are descended from one of two males. *Genomics* 7,325–330.
- Oosthuizen CJ, Herbert JS, Vermaak LK, Brusnicki J, Fricke J, du Plessis L and Retief AE (1990) Deletion mapping of 39 random isolated Y chromosome DNA fragments. *Hum Genet* 85,205–210.
- O'Reilly AJ, Affara NA, Simpson E, Chandler P, Goulmy E and Ferguson-Smith MA (1992) A molecular deletion map of the Y chromosome long arm defining X and autosomal homologous regions and the localisation of the HYA locus to the proximal region of the Yq euchromatin. *Hum Mol Genet* 1,379–385.
- Papadimas J, Goulis DG, Giannouli C, Papanicolaou A, Tarlatzis B and Bontis JN (2001) Ambiguous genitalia, 45,X/46,XY mosaic karyotype, and Y chromosome microdeletions in a 17-year-old man. *Fertil Steril* 76,1261–1263.
- Paracchini S, Stuppia L, Gatta V, Palka G, Moro E, Foresta C, Mengua L, Oliva R, Balleca JL, Kremer JA et al. (2000) Y chromosomal DNA haplotypes in infertile European males carrying Y-microdeletions. *J Endocrinol Invest* 23,671–676.
- Paracchini S, Arredi B, Chalk R and Tyler-Smith C (2002) Hierarchical high-throughput SNP genotyping of the human Y chromosome using MALDI-TOF mass spectrometry. *Nucleic Acids Res* 30,6e27.
- Potocki L, Chen KS, Park SS, Osterholm DE, Withers MA, Kimonis V, Summers AM, Meschino WS, Anyane-Yeboah K, Kashork CD et al. (2000) Molecular mechanism for duplication 17p11.2—the homologous recombination reciprocal of the Smith-Magenis microdeletion. *Nat Genet* 24,84–87.
- Prosser J, Inglis JD, Condie A, Ma K, Kerr S, Thakrar R, Taylor K, Cameron JM and Cooke HJ (1996) Degeneracy in human multicopy RBM (YRRM), a candidate spermatogenesis gene. *Mamm Genome* 7,835–842.
- Quintana-Murci L, Krausz C and McElreavey K (2001a) The human Y chromosome: function, evolution and disease. *Forensic Sci Int* 118, 169–181.
- Quintana-Murci L, Krausz C, Heyer E, Gromoll J, Seifer I, Barton DE, Barrett T, Skakkebaek NE, Rajpert-De Meyts E, Mitchell M et al. (2001b) The relationship between Y chromosome DNA haplotypes and Y chromosome deletions leading to male infertility. *Hum Genet* 108, 55–58.
- Qureshi SJ, Ross AR, Ma K, Cooke HJ, Intyre MA, Chandley AC and Hargreave TB (1996) Polymerase chain reaction screening for Y chromosome microdeletions: a first step towards the diagnosis of genetically-determined spermatogenic failure in men. *Mol Hum Reprod* 2, 775–779.
- Reijo R, Lee TY, Salo P, Alagappan R, Brown LG, Rosenberg M, Rozen S, Jaffe T, Straus D, Hovatta O et al. (1995) Diverse spermatogenic defects in humans caused by Y chromosome deletions encompassing a novel RNA-binding protein gene. *Nat Genet* 10,383–393.
- Repping S, Skaletsky H, Lange J, Silber S, Van Der Veen F, Oates RD, Page DC and Rozen S (2002) Recombination between palindromes P5 and P1 on the human Y chromosome causes massive deletions and spermatogenic failure. *Am J Hum Genet* 71,906–922.
- Repping S, Skaletsky H, Brown L, van Daalen SK, Korver CM, Pyntikova T, Kuroda-Kawaguchi T, de Vries JW, Oates RD, Silber S et al. (2003) Polymorphism for a 1.6-Mb deletion of the human Y chromosome persists through balance between recurrent mutation and haploid selection. *Nat Genet* 35,247–251.
- Repping S, van Daalen SK, Korver CM, Brown LG, Marszalek JD, Gianotten J, Oates RD, Silber S, van der Veen F, Page DC et al. (2004) A family of human Y chromosomes has dispersed throughout northern Eurasia despite a 1.8-Mb deletion in the azoospermia factor c region. *Genomics* 83,1046–1052.
- Rives N, Simeon N, Milazzo JP, Barthelemy C and Mace B (2003) Meiotic segregation of sex chromosomes in mosaic and non-mosaic XYY males: case reports and review of the literature. *Int J Androl* 26,242–249.
- Rosser ZH, Zerjal T, Hurler ME, Adojaan M, Alavantic D, Amorim A, Amos W, Armenteros M, Arroyo E, Barbuji G et al. (2000) Y chromosomal diversity in Europe is clinal and influenced primarily by geography, rather than by language. *Am J Hum Genet* 67,1526–1543.
- Rozen S, Skaletsky H, Marszalek JD, Minx PJ, Cordum HS, Waterston RH, Wilson RK and Page DC (2003) Abundant gene conversion between arms of palindromes in human and ape Y chromosomes. *Nature* 423,873–876.
- Sabeti PC, Reich DE, Higgins JM, Levine HZ, Richter DJ, Schaffner SF, Gabriel SB, Platko JV, Patterson NJ, McDonald GJ et al. (2002) Detecting recent positive selection in the human genome from haplotype structure. *Nature* 419,832–837.
- Sanchez JJ, Borsting C, Hallenberg C, Buchard A, Hernandez A and Morling N (2003) Multiplex PCR and minisequencing of SNPs—a model with 35 Y chromosome SNPs. *Forensic Sci Int* 137,74–84.

- Sandberg AA (1985) Clinical aspects of Y chromosome abnormalities. In *Progress and Topics in Cytogenetics*, Vol. 6. Alan R Liss Inc, New York, Part B.
- Saxena R, Brown LG, Hawkins T, Alagappan RK, Skaletsky H, Reeve MP, Reijo R, Rozen S, Dinulos MB, Distechi CM et al. (1996) The DAZ gene cluster on the human Y chromosome arose from an autosomal gene that was transposed, repeatedly amplified and pruned. *Nat Genet* 14,292–299.
- Semino O, Passarino G, Brega A, Fellous M and Santachiara-Benerecetti AS (1996) A view of the neolithic demic diffusion in Europe through two Y chromosome-specific markers. *Am J Hum Genet* 59,964–968.
- Siffroi JP, Le Bourhis C, Krausz C, Barbaux S, Quintana-Murci L, Kanafani S, Rouba H, Bujan L, Bourrouillou G, Seifer I et al. (2000) Sex chromosome mosaicism in males carrying Y chromosome long arm deletions. *Hum Reprod* 15,2559–2562.
- Simoni M (2001) Molecular diagnosis of Y chromosome microdeletions in Europe: state-of-the-art and quality control. *Hum Reprod* 16,402–409.
- Skaletsky H, Kuroda-Kawaguchi T, Minx PJ, Cordum HS, Hillier L, Brown LG, Repping S, Pyntikova T, Ali J, Bieri T et al. (2003) The male-specific region of the human Y chromosome is a mosaic of discrete sequence classes. *Nature* 423,825–837.
- Solari AJ and Rey Valzacchi G (1997) The prevalence of a YY synaptonemal complex over XY synapsis in an XYY man with exclusive XYY spermatocytes. *Chromosome Res* 5,467–474.
- Soudek D, Langmuir V and Stewart DJ (1973) Variation in the nonfluorescent segment of long Y chromosome. *Humangenetik* 18,285–290.
- Speed RM, Vogt P, Kohler MR, Hargreave TB and Chandley AC (1993) Chromatin condensation behaviour of the Y chromosome in the human testis. I. Evidence for decondensation of distal Yq in germ cells prior to puberty with a switch to Sertoli cells in adults. *Chromosoma* 102, 421–427.
- Sun C, Skaletsky H, Birren B, Devon K, Tang Z, Silber S, Oates R and Page DC (1999) An azoospermic man with a de novo point mutation in the Y chromosomal gene USP9Y. *Nat Genet* 23,429–432.
- Sun C, Skaletsky H, Rozen S, Gromoll J, Nieschlag E, Oates R and Page DC (2000) Deletion of azoospermia factor a (AZFa) region of human Y chromosome caused by recombination between HERV15 proviruses. *Hum Mol Genet* 9,2291–2296.
- Thangaraj K, Subramanian S, Reddy AG and Singh L (2003) Unique case of deletion and duplication in the long arm of the Y chromosome in an individual with ambiguous genitalia. *Am J Med Genet A* 116,205–207.
- The Y Chromosome Consortium (YCC) (2002) A nomenclature system for the tree of human Y chromosomal binary haplogroups. *Genome Res* 12, 339–348.
- Tiepolo L and Zuffardi O (1976) Localization of factors controlling spermatogenesis in the nonfluorescent portion of the human Y chromosome long arm. *Hum Genet* 34,119–124.
- Torrioni A, Semino O, Scozzari R, Sirugo G, Spedini G, Abbas N, Fellous M and Santachiara Benerecetti AS (1990) Y chromosome DNA polymorphisms in human populations: differences between Caucasoids and Africans detected by 49a and 49f probes. *Ann Hum Genet* 54,287–296.
- Turner JM, Mahadevaiah SK, Fernandez-Capetillo O, Nussenzweig A, Xu X, Deng CX and Burgoyne PS (2005) Silencing of unsynapsed meiotic chromosomes in the mouse. *Nat Genet* 37,41–47.
- Underhill PA, Shen P, Lin AA, Jin L, Passarino G, Yang WH, Kauffman E, Bonne-Tamir B, Bertranpetit J, Francalacci P et al. (2000) Y chromosome sequence variation and the history of human populations. *Nat Genet* 26,358–361.
- Unnérus V, Fellmann J and De La Chapelle (1967) The length of the human Y chromosome. *Cytogenetics* 6,213–227.
- Vergnaud G, Page DC, Simmler MC, Brown L, Rouyer F, Noel B, Botstein D, de la Chapelle A and Weissenbach J (1986) A deletion map of the human Y chromosome based on DNA hybridization. *Am J Hum Genet* 38,109–124.
- Vogt PH (1996) Human Y chromosome function in male germ cell development. In *Advances in Developmental Biology*. JAI Press Inc. 4, pp 191–257.
- Vogt PH (1998) Human chromosome deletions in Yq11, AZF candidate genes and male infertility: history and update. *Mol Hum Reprod* 4, 739–744.
- Vogt PH (2004) Genomic heterogeneity and instability of the AZF locus on the human Y chromosome. *Mol Cell Endocrinol* 224,1–9.
- Vogt PH (2005) Azoospermia factor (AZF) in Yq11: towards a molecular understanding of its function for human male fertility and spermatogenesis. *Reprod Biomed Online* 10,81–93.
- Vogt PH and Fernandes S (2003) Polymorphic DAZ gene family in polymorphic structure of AZFc locus: Artwork or functional for human spermatogenesis? *APMIS* 111,115–127.
- Vogt P, Keil R and Kirsch S (1993) AZF-function of the human Y chromosome during spermatogenesis. In *Sumner AT and Chandley AC (eds) Chromosomes Today*, Vol. 11. Chapman & Hall, London, pp 227–239.
- Vogt PH, Edelmann A, Hirschmann P and Köhler MR (1995) The azoospermia factor (AZF) of the human Y chromosome in Yq11: function and analysis in spermatogenesis. *Reprod Fertil Dev* 7,685–693.
- Vogt PH, Edelmann A, Kirsch S, Henegariu O, Hirschmann P, Kiesewetter F, Köhn FM, Schill WB, Farah S, Ramos C et al. (1996) Human Y chromosome azoospermia factors (AZF) mapped to different subregions in Yq11. *Hum Mol Genet* 5,933–943.
- Vogt PH, Affara N, Davey P, Hammer M, Jobling MA, Lau YF, Mitchell M, Schempp W, Tyler-Smith C, Williams G et al. (1997) Report of the Third International Workshop on Y Chromosome Mapping 1997. Heidelberg, Germany, April 13–16, 1997. *Cytogenet Cell Genet* 79, 1–20.
- de Vries JW, Hoffer MJ, Repping S, Hoovers JM, Leschot NJ and van der Veen F (2002) Reduced copy number of DAZ genes in subfertile and infertile men. *Fertil Steril* 77,68–75.
- Yen PH (1999) Advances in Y chromosome mapping. *Curr Opin Obstet Gynecol* 11,275–281.
- Yen P (2001) The fragility of fertility. *Nature Genet* 29,243–244.
- Yunis E, Garcia-Conti FL, de Caballero OM and Giraldo A (1977) Yq deletion, aspermia, and short stature. *Hum Genet* 39,117–122.

Received on February 11, 2005; revised on April 15, 2005; accepted on April 22, 2005