HSFY genes and the P4 palindrome in the AZFb interval of the human Y chromosome are not required for spermatocyte maturation

Elsa Kichine1,†, Virginie Rozé2,†, Julie Di Cristofaro3, Daniel Taulier4, André Navarro1,4, Eric Streichemberger1, Fanny Decarpentrie1, Catherine Metzler-Guillema1,5, Nicolas Lévy1,4, Jacques Chiaroni3, Veronique Paquis-Flucklinger6, Florence Fellmann2,7, and Michael J. Mitchell1,*

1Inserm UMR S 910, Faculté de Médecine de Marseille, Université de la Méditerranée, Marseille 13385, France 2Service de Génétique Biologique-Histologie—BDR and CECOS, CHU, Besançon 25030, France 3UMR 6578, Anthropologie Bio-culturelle, CNRS, EFS, Université de la Méditerranée, Faculté de médecine Hôpital Nord, Marseille 1344, France 4Département de Génétique Médicale, Hôpital d’Enfants de la Timone, AP-HM, Marseille 13385, France 5Laboratoire de Biologie de la Reproduction, Hôpital de la Conception, AP-HM, Marseille 13385, France 6Service de Génétique médicale, CHU Hôpital Archet 2, Nice 06202, France

*Correspondence address. E-mail: michael.mitchell@univmed.fr

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BACKGROUND: Recurrent AZFb deletions on the human Y chromosome are associated with an absence of ejaculated spermatozoa consequent to a meiotic maturation arrest that prevents the progression of germ cells to haploid stages. The extreme rarity of partial deletions has hampered the identification of the AZFb genes required for normal meiotic stages. The critical interval, refined by two overlapping deletions associated with full spermatogenesis (AZFc and b1/b3), measures over 4 Mb and contains 13 coding genes: CDY2, XKRY, HSFY1, HSFY2, CYORF15A, CYORF15B, KDM5D, EIF1AY, RPS4Y2 and four copies of RBMY.

METHODS AND RESULTS: We screened 1186 men from infertile couples for Y chromosome deletions, and identified three unrelated oligozoospermic men and one azoospermic man who carry an identical 768 kb deletion resulting in loss of the entire P4 palindrome, including both HSFY genes, the only coding genes within the deletion interval. This 768 kb deletion was not found in 1179 control men. The deletion breakpoints share only 4 bp of nucleotide identity, revealing that the deletions are not recurrent, but are descendants of a founding deletion. Confiming this, we find that all four men carry a Y chromosome of the same highly defined haplogroup (R1b1b1a1b) (incidence 30% in Southern France), although further haplotype analyses showed that they were not closely related.

CONCLUSIONS: Although the HSFY deletion is restricted to our infertile group, it has been transmitted naturally over many generations, indicating that HSFY genes make only a slight contribution to male fertility. Importantly, our study formally excludes HSFY genes as the AZFb factor required for progression through meiosis.

Key words: Y chromosome / male infertility / spermatogenesis / gene deletion / heat shock factor Y

Introduction

The role of the human Y chromosome in male fertility is well established and deletion mapping has defined three Azosperma factor (AZF) intervals (AZFa, b and c) that are critical for spermatogenesis (Tiepolo and Zuffardi, 1976; Ma et al., 1992; Reijo et al., 1995; Vogt et al., 1996). For each interval, deletions are mainly homogeneous in extent, and result in the loss of multiple genes (Blanco et al., 2000; Kamp et al., 2000; Sun et al., 2000; Kuroda-Kawaguchi et al., 2001; Repping et al., 2002). The extreme rarity of confirmed smaller single...
gene or gene family deletions has largely prevented progress towards determining the contribution of individual Y chromosome genes to human male fertility.

One exception is in the AZFa interval which contains two genes, USP9Y and DDX3Y (Sun et al., 1999). The absence of both AZFa genes leads to a severe phenotype of azoospermia, with testes of reduced volume lacking any germ cells (Vogt et al., 1996). Five partial AZFa deletion patients have been described, however, each with a different deletion affecting the expression of USP9Y uniquely (Sargent et al., 1999; Sun et al., 1999; Krausz et al., 2006; Luddi et al., 2009). In all these latter cases, the spermatogenic process is complete, and in three cases there was natural transmission of the deleted Y chromosome, with transmission over three generations in one family (Krausz et al., 2006). Furthermore, one USP9Y-deleted patient with mild asthenozoospermia met all other criteria of normospermia (Luddi et al., 2009). It has been concluded from these studies that in the absence of USP9Y, spermatogenic efficiency is reduced slightly, allowing normally secondary factors to become preponderant in determining fertility (Tyler-Smith and Krausz, 2009).

Deletion of the AZFb interval is also most often associated with azoospermia, but arising from a maturation arrest during meiosis (Vogt et al., 1996; Krausz et al., 2000). The smallest AZFb deletion (P5/proximal-P1) is typically 6.2 Mb in size and leads to the loss of at least 18 coding genes (Repping et al., 2002). This deletion interval overlaps with the proximal end of the AZFc interval and includes the AZFc genes, DAZ1 and DAZ2, and one copy each of BPY2, CDY1 and PRY. Since the full AZFc deletion removes all copies of DAZ, CDY1 and BPY2, but is associated with complete spermatogenesis, DAZ, CDY1 and BPY2 genes can be excluded as being critical for spermatocyte progression. Further refinement of the region critical for meiotic maturation has been provided by findings of recurrent b1/b3 deletions, and other recently described deletions around palindrome P3, in fertile men and normospermic men (Repping et al., 2003; Machev et al., 2004; Hucklenbroich et al., 2005; Lynch et al., 2005; Noordam et al., 2011). The more extensive b1/b3 deletion interval contains two of the six copies of RBMY1, both functional copies of PRY (Stouffs et al., 2004), one of three copies of BPY2 and two of the four DAZ genes. Thus, the critical AZFb interval necessary for spermatocyte maturation stretches from the centre of palindrome P5 to the proximal edge of P3 within the RBMY1 cluster, an interval of over 4 Mb containing 13 coding genes.

During routine screening for microdeletions of the Y chromosome, we identified four infertile men who carry a previously unknown deletion around the P4 palindrome, at the proximal end of the AZFb interval. The primary objective of our study was to define the precise extent of these deletions, and thus refine mapping of AZFb functions critical for meiotic progression. We showed that the four men carry an identical deletion that has resulted in the loss of both HSFY genes. We therefore also investigated the degree to which the HSFY genes contribute to human male fertility.

Materials and Methods

Patients and controls

The patients were recruited from couples consulting for infertility (n = 1186). They were men who had been diagnosed as oligozoospermic or azoospermic following semen analysis, and referred for AZF interval microdeletion analysis of the Y chromosome. Patients with classic AZF deletions were excluded. Patients with a 47,XXX karyotype were excluded, while other chromosomal anomalies were included. Patients were from Marseilles, Nice and Besançon and the majority had sperm counts below 5 x 10^6 spermatozoa/ml (Table I). Controls (n = 1179) were from Marseilles and Besançon. The control group from Marseilles (n = 1028) consisted of fertile men with at least two children (n = 672), fertile men with at least one child (n = 188) and men of unknown fertility (n = 168). The control group from Besançon (n = 151) consisted of 111 men with at least one child and 40 normospermic men (according to the full WHO criteria). All patients and controls included in the study gave their informed consent, and necessary approvals were obtained from local ethical committees.

Table I Composition of the infertile group by recruitment centre and sperm count.

<table>
<thead>
<tr>
<th>Centre</th>
<th>Sperm concentration – 10^6</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>spermatozoa/ml</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>&lt;5</td>
</tr>
<tr>
<td>Marseilles</td>
<td>96</td>
<td>165</td>
</tr>
<tr>
<td>Besançon</td>
<td>157</td>
<td>373</td>
</tr>
<tr>
<td>Nice</td>
<td>80</td>
<td>68</td>
</tr>
<tr>
<td>Total</td>
<td>333</td>
<td>606</td>
</tr>
</tbody>
</table>

Clinical details of HSFY-deleted men

Clinical details of the HSFY-deleted men are summarized in Table II. Y133 (Nice) was azoospermic. There was no family history of male infertility. He had a normal karyotype, and hormone levels were in the normal range: FSH = 8.9 IU/l; LH = 5.7 IU/l and testosterone = 16.6 nmol/l.

BES301 (Besançon) presented with an oligoasthenoteratozoospermia and a sperm concentration of 3.6 x 10^6/ml. He had a left varicocele (bilateral) and reduced testicular volume (4 ml). Karyotype analysis showed that he carries a balanced translocation t(7;14)(q31;q21). Hormone levels were in the normal range: FSH = 7 IU/l; LH = 2.4 IU/l; testosterone = 18 nmol/l and prolactin = 5.4 mg/l. The most frequent morphological abnormalities were small heads and/or malformed heads of the acrosome.

BES208 (Besançon) presented with an oligonecrototeratozoospermia. A series of nine semen analyses between the ages of 27 and 37 years old showed a decline in sperm concentration from 14 x 10^6/ml to 1 x 10^6/ml. Testes volumes were slightly below normal. His karyotype was normal. FSH levels were normal. He is father to a daughter conceived naturally after 3 years of trying. He was 28 years old at the time of conception (sperm count 6 – 14 x 10^5/ml). Through ICSI, he also fathered a boy and a girl following a twin pregnancy. The most frequent morphological abnormalities were small heads and/or malformed heads of the acrosome.

The patient 13-2883 (Marseilles) presented with complete teratozoospermia. The tails of all spermatozoa were short and some showed thickening of the mitochondrial and fibrous sheath. Sperm concentrations were 1.7 – 9.6 x 10^5/ml. There was testicular hypotrophy that was pronounced on the left and slight on the right. Karyotype analysis showed that the patient carries a pericentric inversion of chromosome 5; 46,XY,inv(5)(p13;q13). Embryos were obtained by IVF but no pregnancy resulted. Embryos were also conceived by ICSI but again no pregnancy resulted. He has a brother who has two daughters.
Sequence-tagged sites mapping

The approximate extent of the deletion was first mapped with previously described markers: CDY2, P5 spacer proximal, P5 spacer distal, P5/P4 junction, sY117 (HSFY), P4 distal boundary, sY2603 and YAP (Vollrath et al., 1992; Hammer and Horai, 1995; Repping et al., 2002; Longejeied et al., 2010) (Fig. 1). The fine mapping of the distal breakpoint from 560 kb (sY2603 to YAP) down to 8 kb was conducted in steps with markers chosen to divide the remaining candidate region into 4–6 intervals. The details of all new markers are provided in Supplementary data, Table S1.

Isolating the HSFY deletion junction fragment

Fifteen micrograms of genomic DNA from the patient 13-2883 was digested with 80 units of BsrGI (New England Biolabs) for 4 h and ligated to EcoRI adaptors at 20°C overnight. The DNA was then precipitated with 0.5 M NaCl and 0.7 volumes isopropanol (30 min, 20°C), washed in 70% ethanol and resuspended in Tris–EDTA (TE) pH 8.0 and migrated overnight on a 0.6% standard agarose gel (Invitrogen). DNA fragments of 9–11 kb were excised from the gel after ethidium bromide staining, and extracted with the freeze-fracture phenol method. Briefly, the gel slice was forced through a 26 G syringe needle, mixed with an equal volume of buffered phenol pH 8.0 and frozen at -80°C. After thawing, the emulsion was centrifuged at 13 000 rpm for 5 min and the upper aqueous phase was recovered and extracted once with one volume of buffered phenol/chloroform (1:1). DNA was precipitated with 0.3 M sodium acetate pH 5.2 and 0.7 vol isopropanol at 20°C overnight. Recovered DNA was resuspended in 3 μl TE and 1.5 μl ligated to EcoRI-digested bacteriophage lambda insertion vector λNM1149, overnight at 16°C. The resulting library (300 000 recombinant phage) was plated on XL1-Blue MRF' (Stratagene). Screening with the AZFb marker (TSNAplex system (Applied Biosystems). The STR polymorphisms on the Y chromosome were determined with the AmpFISTR Yfiler PCR Amplification Kit (Applied Biosystems). The STR loci DYS385 did not amplify and could not be scored because they are situated in P4 between the HSFY genes deletion interval.

The HSFY genes were sequenced on Y chromosomes of different haplogroups by amplification and sequencing with oligonucleotides o3619 and o3620 (exon 1), o3621 and o3622 (exon 2) and o3623 and o3624 (exon 7). Primer sequences are provided in Supplementary data, Table S1.

Table II Karyotypes and sperm parameters of the four HSFY-deleted patients.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Karyotype</th>
<th>Semen volume (ml)</th>
<th>Sperm count ($10^3/ml$)</th>
<th>Motility (a/b)</th>
<th>Normal forms (%)</th>
<th>Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y-133</td>
<td>46,XY</td>
<td>2.5</td>
<td>0</td>
<td>—</td>
<td>—</td>
<td>Azoospermia</td>
</tr>
<tr>
<td>13-2883</td>
<td>46,XY,inv(5)(p13,q13)</td>
<td>0.7–1.1</td>
<td>1.7–9.6</td>
<td>0/20 0/10</td>
<td>22 0</td>
<td>Flagella short and thick</td>
</tr>
<tr>
<td>BES301</td>
<td>t(7;14)(q31;q21), balanced</td>
<td>3–5</td>
<td>0.9–9.6</td>
<td>0/5 5/15</td>
<td>11 16</td>
<td>Head and acrosome abnormalities</td>
</tr>
<tr>
<td>BES208</td>
<td>46,XY</td>
<td>—</td>
<td>14 0/0.9</td>
<td>20 15 5/15</td>
<td>49 14</td>
<td>Head and acrosome abnormalities</td>
</tr>
</tbody>
</table>

$\gg$ indicates an evolution over time.

aWHO criteria.
bDAVID classification.

Results

During routine screening for Y microdeletions in infertile men, we identified three men lacking proximal AZFb markers from palindrome P4: sY117, a marker with one copy in each HSFY gene, and sY114, a marker flanking the central P4 spacer. A fourth case was identified by specifically screening for deletions of HSFY in 150 oligozoospermic and azoospermic patients from Nice. Clinical details of these four patients are summarized in Table II. We did not, however, detect this deletion in 1179 control men. We further defined breakpoints using additional sequence-tagged sites (STS) markers: the proximal breakpoint mapped to the distal arm of palindrome P5 and could not be mapped further using STS markers, because of the presence of the entire proximal arm, while the distal breakpoint was mapped 73–81 kb distal to P4 within an 8 kb interval containing a human endogenous retroviral element (Fig. 1). By Southern analysis, we identified and isolated a 10.5 kb BsrGI restriction fragment that spans the junction of the deletion breakpoints in patient 13-2883. Sequencing of this fragment defined the precise deletion breakpoints, revealing the deletion to extend 767 713 bp from 40 kb distal to XKRY in the distal arm of P5 to a position 73 kb distal to P4 (Fig. 1 and Human genome assembly GRCh37/hg19: chrY:20338212–21105928). The entire 419 kb P4 palindrome is deleted.

The identical STS results for all four patients suggested that they all carry the same deletion. We tested this by PCR with primers (o3575/o3576) from each side of the junction defined in patient 13-2883. A fragment of 750 bp was amplified from each of the four patients deleted for HSFY, but not from control men without the deletion (Fig. 1). Sequencing the amplified junction confirmed that the four infertile men lacking HSFY carry an identical deletion. The deletion results in the loss of eight transcribed units from the Y chromosome: the coding genes, HSFY1 and HSFY2 and the non-coding units

Y chromosome haplogroups and short tandem repeat haplotypes

Haplogrouping for assignment to the major branches of the Y chromosome haplogroup tree (Karatet al., 2008) was carried out as previously described (Maidie et al., 2010). High resolution haplogrouping of R-M207 was performed using direct sequencing of multiplexed fragments with the SNaPshot multiplex system (Applied Biosystems), as previously described (Myres et al., 2010). Alleles of 15 short tandem repeat (STR)
Figure 1  Defining the extent of the HSFY gene deletion interval within the AZFb interval of the human Y chromosome. (A) The AZFb, AZFc and b1/b3-deletion intervals are represented based on published maps (Repping et al., 2002). All coding genes are shown, but of the five non-coding transcription units deleted with HSFY, only TTTY14 is shown (see Table III for a full list). Key STS markers are shown as empty (absent) or filled (present) circles: 1, CDY2; 2, proximal P5 spacer; 3, distal P5 spacer; 4, P5/P4 junction; 5, y117 (HSFY) and y114; 6, P4 distal boundary; 7, y2603; 8, o1798/o1799 (TTY14); 9, o2041/o1765; 10, YAP. (B) The HSFY deletion junction fragment was identified by Southern analysis of genomic DNA from M, control male, patient 13-2883 and F, female, using the STS marker o2041/o1765 as probe. A specific junction fragment was revealed with the restriction enzymes BsrGI and XmnI. The 10 kb BsrGI fragment specific to 13-2883 was isolated and the junction sequenced. (C) Using primers o3575/o3576 that flank the junction, a fragment of 750 bp was amplified by PCR from the genomic DNA of the four HSFY-deleted men (13-2883, BES208, BES301 and Y-133), but not M, a control male. (D) Sequencing of the 750 bp fragment amplified from the four HSFY deletion cases revealed an identical junction between P5 and TTTY14. The four bases of sequence identity between the two extremities of the deletion are highlighted in grey.
Table III  The copy number of AZFb genes and transcription units on normal and deleted Y chromosomes.

<table>
<thead>
<tr>
<th>Transcription unit</th>
<th>Coding</th>
<th>Copy number</th>
</tr>
</thead>
<tbody>
<tr>
<td>CDY2</td>
<td>Yes</td>
<td>2</td>
</tr>
<tr>
<td>XXRY</td>
<td>Yes</td>
<td>2</td>
</tr>
<tr>
<td>NCRNA00230</td>
<td>No</td>
<td>2</td>
</tr>
<tr>
<td>FAM41AY</td>
<td>No</td>
<td>2</td>
</tr>
<tr>
<td>TTTY9</td>
<td>No</td>
<td>2</td>
</tr>
<tr>
<td>HSFY</td>
<td>Yes</td>
<td>2</td>
</tr>
<tr>
<td>NCRNA00185</td>
<td>No</td>
<td>1</td>
</tr>
<tr>
<td>TTTY14</td>
<td>No</td>
<td>1</td>
</tr>
<tr>
<td>CYORF15A</td>
<td>Yes</td>
<td>1</td>
</tr>
<tr>
<td>CYORF15B</td>
<td>Yes</td>
<td>1</td>
</tr>
<tr>
<td>KDM5D</td>
<td>Yes</td>
<td>1</td>
</tr>
<tr>
<td>TTTY10</td>
<td>No</td>
<td>1</td>
</tr>
<tr>
<td>EFI1AY</td>
<td>Yes</td>
<td>1</td>
</tr>
<tr>
<td>RPS4Y2</td>
<td>Yes</td>
<td>1</td>
</tr>
<tr>
<td>TTTY13</td>
<td>No</td>
<td>1</td>
</tr>
<tr>
<td>RBMY1</td>
<td>Yes</td>
<td>6</td>
</tr>
</tbody>
</table>

The del-HSFY interval units are shown in bold. The P5/proxP1 deletion is the full AZFb deletion.

NCRNA00185, TTTY14, FAM41AY2, NCRNA00230B and TTTY9A and B. A comparison with the full AZFb deletion is presented in Table III.

There is an identity of only four nucleotides (CTAC) flanking the deletion interval, implying that the deletions are unlikely to have occurred independently through non-allelic homologous recombination, but are instead descendants of a founding deletion. Consistent with a founder effect, we determined that each man carries a Y chromosome of the same highly resolved haplogroup, R1b1b1a1-S116*(xU152,M529) (Fig. 2). This paragroup has been found with highest frequencies in Spain (50%) and in the South of France (30%) (Myres et al., 2010).

Although the P4-deleted men do not share surnames, and are therefore unlikely to be closely related, we verified this by haplotyping their Y chromosome at 15 STR loci (Table IV). The duplicated DYS385 loci could not be scored because they map within the HSFY-deletion interval. At the 15 STR loci scored, we found differences at five loci with 3–4 loci differing between individuals predicting 1–2 mutations per lineage. Assuming an average mutation rate of 2.4 × 10⁻³ per generation per locus (Goedbloed et al., 2009), one difference at 15 loci would be expected every 28 generations. It is therefore clear that the men are not close relatives, and that the P4 deletion has been transmitted over multiple generations. This establishes that HSFY and the non-coding units, NCRNA00185, TTTY9 and TTTY14, are not absolute requirements for human male fertility; they have, at most, only a slight positive impact on human male fertility.

To gain further insights into whether HSFY1 and HSFY2, the only coding genes within the deleted interval, are necessary for full human fertility, we investigated their conservation during human evolution. It has recently been determined that the coding regions of the 16 single copy ‘X-degenerate’ genes on the Y chromosome have been conserved in all major branches of the Y chromosome haplogroup tree. HSFY, being present in two copies, was not included in this analysis (Rozen et al., 2009). We therefore determined the sequence of the HSFY genes in 12 of the major branches of the Y chromosome haplogroup tree (Karafet et al., 2008) (haplogroups A, B, C, E-M2, E-M35, G, H, I, J, L, N and R1b-S116(xU152,M529,del-HSFY) (Fig. 3). We sequenced the three HSFY exons that compose the two HSFY transcript variants designated as protein coding in the Entrez Gene database at National Center for Biotechnology Informations (NM_153716—variant 1 and NM_001001877—variant 2).
These have previously been numbered as exons 1, 2 and 7 (Tessari et al., 2004). Only a single nucleotide polymorphism was detected, in the haplogroup A branch, a silent substitution in exon 2 of one of the two HSFY genes (T699A in transcript variant 1). Thus functional HSFY genes have been conserved on the Y chromosome for 100,000 years during the evolution of modern humans, indicating that HSFY does make a real, if modest, contribution to the reproductive fitness of men.

Discussion

Here, we describe a deletion variant of the haplogroup R1b1b1a1-S116 human Y chromosome that is exclusive to our infertile sample. Compared with the reference Y chromosome, it lacks the two identical coding genes HSFY1 and HSFY2, and six non-coding transcription units FAM41AY2, NCRNA00230B, TTTY9A, TTTY9B, NCRNA00185 and TTTY14. FAM41AY2 and NCRNA00230B are localized in the distal arm of P5 and intact copies of each remain in the proximal arm. No copies remain of the other transcribed units. A larger AZFb deletion has previously been described in an azoospermic man, that removes the HSFY genes and three additional coding genes, KDM5D, CYORF15A and CYORF15B (Vinci et al., 2005). Testicular histology was not available in this
latter case however, preventing us from drawing any conclusions regarding the role of these three genes in spermatocyte maturation. On the contrary, the absence of all copies of the HSFY, TTY9, NCRNA00185 and TTY14 genes from the three oligozoospermic men reported here formally excludes them as AZFb factors required for meiotic progression.

One indication that this deletion is not a neutral variant comes from the fact that results in the loss of the entire 419 kb palindrome, P4. There are 11 palindromic structures on the Y chromosome (P1–P8 and IR1–IR3), and these are inferred to contribute to spermatogenic efficiency because, with the exception of TSPY, they contain all the Y-borne testis-specific protein-coding gene families (Skaletsky et al., 2003). HSFY1 and HSFY2 are the only coding genes within P4.

HSFY is expressed specifically in the testis, and immunohistochemical analysis has localized the HSFX protein to the nuclei of most germ cells and the cytoplasm of Sertoli cells (Shinka et al., 2010). HSFY shows homology to the heat shock transcription factor-type (HSF) DNA-binding domain. HSFX does not however bind to heat shock elements (Shinka et al., 2004), and no HSFX-targeted promoters have been identified during spermatogenesis.

HSFY genes appear to have played an important role in males during the evolution of therian mammals because orthologues of the human HSFY gene have been found on extant metatherian (marsupial) and eutherian (placental) Y chromosomes (Murphy et al., 2003; King et al., 2005; Lynch et al., 2005; Jobling et al., 2007; Noordam et al., 2011). Although we have tested large numbers of patients and controls, our finding of only four individuals in the infertile group who carry this 768 kb deletion means that the difference with the control group is not statistically significant (P = 0.063, Fisher’s exact test, one-sided). It therefore remains formally possible that the P4 deletion is a rare neutral polymorphism with no effect on fertility. Against this is the strong evidence that a functional copy of HSFX has been selectively retained on the Y chromosome during the evolution of therian mammals, and our demonstration that HSFX genes have remained practically unchanged during the evolution of modern humans. Furthermore, human genetic diversity studies, using Y chromosome STR markers located in the three AZF intervals, have identified men with full AZFa (n = 1), b (n = 1) and c (n = 3) deletions (Ballard et al., 2005; King et al., 2005), despite the absence of infertility as a recruitment criterion. In contrast, no specific deletions of the widely used P4 marker DYS385, as described here for the HSFX-deleted men, have been reported in such studies (Roewer et al., 2001; Goedbloed et al., 2009), confirming that isolated deletions of both HSFX genes are extremely rare in men throughout the world.

Our data show that there has been transmission of an HSFX-deleted Y chromosome over many generations. This infers that the deletion has at most only a slight negative impact on male fertility, and that this must have been compensated for by environmental, genetic or social factors. One possibility is that the deletion has late-onset effects, which are more pronounced today because of the increasing age at which men become fathers. In this regard, it is of interest that I3-2883 and BES208 manifested a decline in their sperm quality over time (Table II). A further possible explanation is suggested by the fact that two of the HSFX-deleted men (BES301 and 13-2883) have a cytogenetic anomaly that is likely to reduce sperm production impact on fertility, since each transmitted USP9Y deletion remains specific to one family, and none have been found in unrelated men (Krausz et al., 2006; Luddi et al., 2009). Partial AZFc deletions are found at high frequencies in the general population (2–50%) and even in the normospermic population (0.4–19%) (Repping et al., 2003; Giachini et al., 2005; Hucklebroich et al., 2005; Ravel et al., 2006). However, several studies have found that gr/gr deletions are associated with a significant risk of spermatogenic failure (Repping et al., 2003; Ferlin et al., 2005; Giachini et al., 2005). Thus, like partial AZFc deletions, the HSFX provides an example of a Y chromosome variant that paradoxically appears to reduce fertility without being eliminated from the population.

It is noteworthy that USP9Y and HSFX, whose deletions appear to have only a slight negative impact on human male fertility (Luddi et al., 2009), are also two human X-degenerate genes that do not have a functional orthologue on the chimpanzee Y chromosome (Hughes et al., 2005, 2010). This indicates that HSFX, and USP9Y, have made only a limited contribution to reproductive fitness during the evolution of hominids (great apes). Conversely, it may also mean that a limited contribution to male fertility is made by CYorf15B, TBL1Y, TMSB4Y, PRR and XKRY, the other five human Y coding genes with no orthologue in chimpanzee (Hughes et al., 2010). This does appear to be true for the TBL1Y gene and the two PRR genes, each of which is absent on rare variant Y chromosomes, in either non-mospermic men or in men who were not selected on the basis of infertility (Repping et al., 2003; Machev et al., 2004; Hucklebroich et al., 2005; Lynch et al., 2005; Jobling et al., 2007; Noordam et al., 2011).
by increasing the numbers of germ cells that fail to complete meiosis because of pairing difficulties. Thus, the loss of HSFY expression alone might not greatly increase the chances of male infertility in the absence of other risk factors, such as age, chromosomal anomalies, environmental effects or reduced female fertility. The patient 13-2883 has in addition a specific phenotype of immotile spermatozoa with a short flagellum that was not present in other HSFY-deleted patients, or presumably in his fertile father and brother (no semen or DNA samples available), suggesting that this sterilizing phenotype may not be linked to the loss of HSFY. Nevertheless, the absence of HSFY could influence the phenotypic expression of certain allelic variants of genes involved in flagellar formation, either directly, or through a negative effect on the environment of the seminiferous tubule.

The P4/HSFY deletion described here represents an interesting new paradigm with the potential to refine our understanding of how the Y chromosome contributes to human fertility. The link between the P4/HSFY deletion and reduced fertility can now be verified by screening large numbers of patients and controls by centres around the world. Based on our clinical findings for four men carrying this deletion, we would recommend testing for P4/HSFY deletion not only in patients with low sperm counts recruited for Y microdeletion screening, but also in patients with sperm counts higher than 5 million/ml, even if an aetiology of infertility (genetic or not) has already been determined. The HSFY loci are ideally placed to detect deletions of proximal AZFb and as such can easily be incorporated into a routine Y microdeletion screening strategy. Our study further suggests that careful screening of patients for deletions of each gene on the Y chromosome will detect rare, non-recurrent, geographically limited variants that should ultimately allow the identification of the Y chromosome genes that are indispensable for human male fertility.

Supplementary data

Supplementary data are available at http://humrep.oxfordjournals.org/.

Web resources


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Authors’ roles

M.J.M. and F.F. were involved in study conception. E.K., V.R., D.T., A.N., E.S., F.D. and M.J.M. played a role in molecular detection and characterization of deletions, screening of patients, and controls and sequencing of HSFY genes; experimental design and interpretation. J.D.C. and J.C. contributed to fine haplologrouping of the Y chromosome. C.M.G., N.L., J.C., V.P.F. and F.F. were involved in recruitment of patients and controls, collection and analysis of clinical data. M.J.M. and F.F. took direct responsibility for the manuscript. All authors contributed to the writing and revision of the manuscript.

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Conflict of interest

None declared.

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