USP26 gene variations in fertile and infertile men


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BACKGROUND: The human X chromosome is enriched with testis-specific genes that may be crucial for male fertility. One is the ubiquitin-specific protease 26 (USP26). Five frequent mutations have been identified: 1737G>A, 1090C>T, 370–371insACA, 494T>C and 1423C>T (with the latter three usually detected in a cluster). Their role in infertility is still controversial. This study assesses the association of the most frequent USP26 mutations with male infertility and male infertility etiology factors.

METHODS: The study included 300 infertile and 287 fertile men. Data were collected on ethnicity (according to maternal origin) and family history of reproduction. Clinical records from 235 infertile and 62 fertile (sperm bank donors) men were available and summarized. The five mutations were investigated by bioinformatic tools and their frequencies were assessed by restriction analysis. The results were correlated with clinical findings. Segregation of the mutations in four families was analyzed.

RESULTS: The five analyzed mutations were detected in 44 men from both fertile and infertile groups. The cluster and the 1090C>T mutations showed the highest frequency among Arabs and Sephardic Jews of the infertile group, respectively. Inheritance studies showed that mutations were not always associated with the infertility trait. Mutations 1090C>T and 1737G>A were significantly associated with a history of inguinal hernia (P = 0.007 and P = 0.043, respectively). The prevalence of inguinal hernia among men with the 1090C>T mutation was 33.3% (5/15 men), higher than that reported in infertile men (6.7%). CONCLUSIONS: Mutation 1090C>T may be a new genetic risk factor for developing inguinal hernia which may be associated with impaired male fertility.

Keywords: USP26; male infertility; inguinal hernia; genetic risk factors; X chromosome; male fertility factors

Introduction

It has been hypothesized that many cases of abnormal spermatogenesis, cryptorchidism, penile malformations and testicular cancer may comprise a testicular dysgenesis syndrome (TDS) with a common origin in fetal life (Sharpe and Skakkebaek, 2008). The hypothesis is that abnormal testis development, which could have numerous primary causes, results in secondary hormonal or other malfunctions of Leydig and/or Sertoli cells during male sexual differentiation, leading to increased risk of reproductive disorders. Male fertility may be impaired by other pathologies, among them varicocele and inguinal hernia. Inguinal hernia is a relatively common disorder that requires surgery (hernioplasty/herniorrhaphy) (Brunicardi, 2005). Hernioplasty for adults consists of reconstruction of the inguinal floor, while herniorrhaphy for children involves closing off the patent processus vaginalis. Both of them are performed in close proximity to the testis and to the spermatic cord which may be accidentally damaged during the operation. The incidence of inguinal hernia in the Israeli infertile population was reported as being 6.65% (Yavetz et al., 1991).

More than 3000 genes are involved in the genetic network required for male fertility as inferred from studies on mouse and Drosophila genes knock-out mutants (Vogt, 2004). Recent studies have been mainly focusing on genes with a testis-specific expression pattern. The human X chromosome is enriched with testis-specific genes that may be crucial for male fertility (Wang et al., 2001). One of them is the ubiquitin-specific protease 26 (USP26), located at Xq26.2. It is comprised of a single exon encoding for a protein of 913 amino acids. USP26 protein belongs to the family of deubiquitinating enzymes (DUBs), which play an important role in a wide range of cellular processes, such as cell cycle progression, signal transduction, protein quality control, transcriptional regulation and growth control (Amerik and Hochstrasser, 2004). These enzymes are responsible for processing inactive ubiquitin precursors, proofreading ubiquitin–protein conjugates and removing ubiquitin from cellular adducts (Nijman et al., 2005). During spermatogenesis, DUB enzymes might be involved in processes such as the removal of histones and the regulation of protein turnover during meiosis (Baarends et al., 2001).
In mice, USP26 mRNA is present through all the stages of spermatogenesis, with a decreased amount during meiosis apparently due to X-inactivation (Wang et al., 2005). Its expression was reported to be testis-specific in mice and humans (Wang et al., 2001) and it was recently shown to be expressed in human ovary as well, at a level 10-fold lower than in the testis (Kosłowski et al., 2006).

Several sequence changes in the USP26 gene were detected among infertile men (Paduch et al., 2005; Stouffs et al., 2005). Three mutations, usually found to be clustered in the same allele, were detected in 12 men with Sertoli cell-only (SCO) syndrome and in one man with maturation arrest. The cluster mutations were 370–371insACA, 494T > C and 1423C > T, and they led to the amino acid changes T123–124ins, L165S and H475Y, respectively. These mutations were not detected in two studies with a total of 169 control samples (Paduch et al., 2005; Stouffs et al., 2005). Five of the 12 men carrying the cluster mutations were of Arabic origin (Middle East) and two were Caucasians, while the ethnic origins of the remaining five subjects were not recorded. The authors concluded that this haplotype (370–371insACA, 494T > C, 1423C > T) may either cause infertility or be a predisposing factor. In another study on 146 oligozoospermic or cryptozoospermic men and 202 control men from Caucasian origin, however, the cluster mutation was also identified in one man from the control group (Stouffs et al., 2006).

Ravel et al. (2006) further investigated the potential role of the cluster mutations by analyzing 1433 men from different geographic origins. They found that the clustered mutations are compatible with fertility. Furthermore, analysis of a chimpanzee sequence indicated that two of the changes (T123–124ins, L165S) are present in primates and actually represent the ancestral state rather than a derived mutation. Their study demonstrated significant frequencies of the cluster mutations in USP26 in sub-Saharan, South-East Asian and South Asian populations. Interestingly, an additional compound mutation that included the 370–371insACA mutation and a new change (466G > A) was recently reported in nine Chinese oligozoospermic men (Zhang et al., 2007). Paduch et al. (2005) demonstrated two additional changes, 1090C > T and 1737G > A, leading to the amino acid changes L364F and M579I, with frequencies of 4.3 and 1.6%, respectively, but they were not investigated in greater depth.

In order to clarify the role of the observed sequence variants in male infertility and TDS, we investigated the five most frequent changes (370–371insACA, 494T > C, 1423C > T, 1090C > T and 1737G > A) by bioinformatic tools and assessed their frequency in 587 Israeli men with known fertility status. The ethnic origin of 498 men was also known. We compared our results with clinical findings and also analyzed the inheritance of mutated USP26 in four families.

Materials and Methods

Bioinformatics

The sequence from NCBI (NM_031907.1) was used as the reference sequence. Homologs of the USP26 gene were taken from the Swiss-Prot database, and Blast software was used for analysis. Multiple sequence alignments were achieved by Muscle software, and consensus and motifs sequences were found with Prosite software. Three-dimensional (3D) prediction of USP26 normal and mutant proteins was done with NEST software using USP7_Human (1nb8: a PDB) protein as a template. Proteins superposition was done with Multiprof software and viewed with RasTop software.

Participants

The 587 study participants were divided into two groups according to their fertility status. The ethnicity of 498 men was known and it was categorized according to the mother’s origin because USP26 is located on chromosome X. The Arabs were Moslem, Christian and Druze men. The Sephardic Jews were all the Jewish men who were not of Ashkenazi origin. The control group included 287 men with proved fertility of whom 62 were sperm bank donors and 225 were known fertile men with at least 2 children. All the 62 sperm donors were normozoospermic (sperm concentration above 40 × 10⁹; sperm motility >50% at first hour; normal morphology >14%), had normal karyotype, no AZF microdeletion and no clinical history of varicocele, cryptorchidism or inguinal hernia. They were not analyzed separately because they comprised a relatively small group that was subdivided according to their ethnicity and did not have sufficient statistical power. None of the 225 known fertile men had a AZF microdeletion. They were not karyotyped or clinically evaluated. The infertile group included 300 men, of whom 222 (74%) were azoospermic, 57 (19%) were oligozoospermic and 21 (7%) were infertile with unknown semen parameters. There were 20 men (6.6%) who had AZF deletions: 13 complete AZFc, 2 AZFb (P5/proximal P1), 2 AZFc and 3 AZFa-c. Clinical records of 235 men were available. Of the 165 men who underwent karyotype analysis, 146 (89.5%) had a normal karyotype, 3 had the polymorphic inversion on chromosome 9, 7 had Klinefelter syndrome (47XXY), 5 had a mosaic karyotype (<15% of the cells had an abnormal karyotype) and 1 had a 47XXY karyotype. The testis size of 240 infertile men was available. Testosterone levels were reported in 178 clinical records and classified as normal, low or high according to the normal range reported by each laboratory.

We analyzed familial inheritance of the mutations in four families. Two infertile men consented to involve their families, and their fertile brothers and fathers consented to participate in the study. The fathers were included in the control group (different X chromosome than the propositus), but the brothers were not. Two other men with the mutations had two brothers treated in our clinic, and both consented to participate in our study.

All the study participants consented to undergo genetic evaluation. This study was approved by the local Institutional Review Board committee in accordance with the Helsinki Declaration of 1975.

Testicular tissue evaluation

Testicular tissues from the 191 patients who underwent testicular sperm extraction (TESE) were histologically and cytologically evaluated as previously described (Kleiman et al., 2001, 2004). Accordingly, 27 (14%) men had normal spermatogenesis, 90 (47%) had hypospermatogenesis, 21 (11%) had complete spermatocyte maturation arrest and 53 (28%) had SCO.

Mutations analysis

DNA from all subjects was extracted from peripheral blood lymphocytes using MasterPure™ Genomic DNA Purification Kit for blood (Epicentre, WI, USA).

The 370–371insACA mutation was analyzed by 10% acrylamide gel, while the 494T > C, 1423C > T, 1090C > T and 1737G > A...
mutations were detected by restriction analysis performed on the PCR products and analyzed on an agarose gel (2–4%) (Table I, Supplementary data, Fig. S1). A mismatch in the forward primer was introduced for distinguishing between the normal allele and the mutant allele on position 1737 by restriction with FokI. The restriction sites that distinguish between the normal and mutant allele were identified with the help of three web sites (http://cedar.genetics.soton.ac.uk/public_html/prime_r.html, http://www.genscript.com/cgi-bin/tools/ enzyme_cutter.html and http://www.justbio.com/cutter/index.php).

Microsatellite analysis

Four microsatellites were analyzed by ABI PRISM GeneScan using fluorescent primers labeled with FAM or NET. Markers ZS2788 and ZS3736 were 3.5 and 4 Mb upstream from the USP26 gene and markers ZS2951 and ZS2629 were 2 Mb downstream, respectively.

Statistical analysis

Fisher’s exact test assessed the association between the sequence variants and fertility status, familiar infertility and clinical parameters. Student’s t-test assessed the significance of testicular volume between the groups. All statistics were performed at the Statistical Department of Tel Aviv University.

Results

Bioinformatic analysis of the reported mutations

Five mutations previously identified in the gene USP26 were analyzed with bioinformatic tools to assess their possible effect on the USP26 protein. We used multiple sequence alignments of the USP26 predicted protein with its homologues (paralogues and orthologues) proteins and revealed that positions 122, 364 and 579 were evolutionarily preserved, and that the amino acids substitutions were conservative (Table II). Less evolutionarily preserved (i.e. having non-conservative substitutions) were positions 124 and 165, while positions 123 and 475 were hardly preserved.

Three putative phosphorylation motifs were identified: two (TQK and TQKE) started at position 123 and the third (RKMT) started at position 577. Insertion of an additional T at position 123 (370–371insACA mutation) simply moved (RKMT) started at position 577. Insertion of an additional T (TQK and TQKE) started at position 123 and the third positions 123 and 475 were hardly preserved.

A (M579I) abolished the third motif.

Lastly, we assessed the impact of the mutations in the 3D USP26 structure as predicted by NEST software using the reported 3D structure of the far phylogenetic homologue USP7. Only the 3D structure of the USP domain was predicted because the similarities among the USP genes are almost exclusively in this region. Mutations L364F, H475Y and M579I—all three found in the domain region—were analyzed by superposition with the Multi Prot software (Fig. 1). The 3D structure was clearly disturbed by mutation H475Y but not by the amino acid changes in the other two positions.

Mutation frequencies in the study groups

The control and the infertile groups had similar ethnic distributions (Supplementary data, Table S1). The five analyzed mutations were detected in 44 men from both groups (Table III). Mutations 370–371insACA (T123–124ins), 1423 C > T (H475Y) and 494 T > C (L165S) were always identified together in the same allele and were termed ‘cluster’ mutation. The cluster and 1090 C > T (L364F) mutations were found more frequently among the fertile men than the fertile ones, but the differences in frequency did not reach a level of significance. Mutation 1737 G > A (M579I) was barely detected. Two of the fertile men with mutations had an additional genetic defect: one had an AZFc deletion plus mutation 1090 C > T (L364F) and the other had Klinefelter syndrome (47,XXY) in which one X-allele had the cluster mutation.

Variable histological findings on testicular specimens were observed in the infertile men with the mutations and who underwent TESE. Of the biopsies, 2 showed normal spermatogenesis, 11 showed hypospermatogenesis, 1 had maturation arrest and 3 were SCO. In addition, among the men in the fertile group, mutations 1090 C > T (L364F) and 1737 G > A (M579I) were detected in normozoospermic sperm donors (3 and 1, respectively).

The frequency of mutations varied among the ethnic categories (Table III), with more cluster and 1090C > T mutations having been found among the infertile Arabs and Sephardic Jews. There was no significant differences in the frequency of the mutations between the fertile and infertile men within the ethnic subgroups.

Table I. PCR primers, annealing temperature, product size and fragments sizes after restriction treatment for detection of the USP26 variations

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Primers (5’–3’)</th>
<th>Amplification temperature (°C)</th>
<th>PCR product size (bp)</th>
<th>Restriction enzyme</th>
<th>Restriction fragments (bp)</th>
<th>Mutant allele</th>
<th>Normal allele</th>
</tr>
</thead>
<tbody>
<tr>
<td>370–371 InsACA</td>
<td>Left GACCTGGTAAGGGTGGGAGT Right TCTCATCACAATTGTGGGAAATGAA</td>
<td>60</td>
<td>81</td>
<td>–</td>
<td>303</td>
<td>85, 218</td>
<td></td>
</tr>
<tr>
<td>(T123–124ins)</td>
<td>Left TTGTTGACCTCATGAGCTTG</td>
<td>62</td>
<td>303</td>
<td>FOK1</td>
<td>303</td>
<td>20, 91, 138</td>
<td>20, 91, 436</td>
</tr>
<tr>
<td>1423 C &gt; T (H475Y)</td>
<td>Right TGGAAACTGATAGACTTCCTGGT</td>
<td>62</td>
<td>547</td>
<td>TAQ1</td>
<td>20, 48, 104, 247</td>
<td>104, 295</td>
<td></td>
</tr>
<tr>
<td>494 T &gt; C (L165S)</td>
<td>Left GGGAAACAAAATACCTGCAT Right AAGGATTTTCAGTGGGCCGTTC</td>
<td>56</td>
<td>400</td>
<td>DRAG1</td>
<td>48, 104, 247</td>
<td>36, 160</td>
<td></td>
</tr>
<tr>
<td>1090C &gt; T (L364F)</td>
<td>Left TTCTCAATCCACATTTGGCCT Right GCAATGGAGTGCAACCACTC</td>
<td>54</td>
<td>196</td>
<td>FOK1</td>
<td>196</td>
<td>36, 160</td>
<td></td>
</tr>
<tr>
<td>1737G &gt; A (M579I)</td>
<td>Left CAATTATTTAAAAATGGTATTTCGAAAGGAT Right TTTGGTTTGAATTTTTTCCA</td>
<td>54</td>
<td>196</td>
<td>FOK1</td>
<td>196</td>
<td>36, 160</td>
<td></td>
</tr>
</tbody>
</table>

*The three mutations that were always found in the same allele and called ‘cluster’; The mismatch added to the primer is in bold underline.
The second family was of Sephardic (Libya) Jewish origin and the propositus carried the cluster mutation in USP26. Both brothers were Moslem Arab in whom the presence of infertility was inherited together with the cluster mutation (Fig. 2A). The first family was from Yemen (Sephardic Jewish) and the fourth family was from Libya with infertility. Both parents in the fourth family were Moslem Arabs. The mutation was observed in the father of the propositus and his fertile brother. In addition, four microsatellite markers located in close proximity to the USP26 gene were identical in both brothers (data not shown). The propositus had undergone unilateral right orchidectomy when he was 6 years old and was azoospermic with hypospermatogenesis in the testicular tissue.

**Inheritance of USP26 mutations**

Four families were analyzed to further assess whether the mutant alleles were inherited together with infertility. The first was a Moslem Arab family in whom the presence of the cluster mutation was analyzed (Fig. 2A). Both brothers had similar testicular findings (spermatocyte maturation arrest), but only the propositus carried the cluster mutation in USP26. The second family was of Sephardic (Libya) Jewish origin and the propositus showed similar findings for mutation 1737G > A (Fig. 2B). The propositus and one of his brothers were azoospermic, although mutation 1737G > A was only detected in the propositus.

Inheritance of mutation 1090C > T was studied in the third and fourth families (Fig. 2C and D, respectively). In the third family, the mother was from Yemen (Sephardic Jewish) and the father was a Moslem Arab. The mutation was observed only in the azoospermic propositus in whom no sperm cells were found in a TESE procedure. He reported a paternal cousin with infertility. Both parents in the fourth family were Moslem Arabs. The mutation was detected in both the infertile propositus and his fertile brother. In addition, four microsatellites located in close proximity to the USP26 gene were identical in both brothers (data not shown). The propositus had undergone unilateral right orchidectomy when he was 6 years old and was azoospermic with hypospermatogenesis in the testicular tissue.

**Clinical and familial findings in men with USP26 mutations**

No significant differences of the testis volume were observed between infertile men without (n = 219; 14.6 ± 7.38 ml) and those with mutations (n = 21; 16.1 ± 7.11 ml) (P = 0.39). The level of testosterone was normal in 83% and 81% of the infertile men without and with mutations, respectively. The difference was not significant (P = 0.0571).

A family history of reproduction was available for 489 men. Analysis of the relationship between familial infertility and the presence of the mutations revealed no significant correlation.

Clinical findings which are more frequently reported among infertile men, such as inguinal hernia, varicocele and cryptorchidism, were tested for association with USP26 mutations (Fig. 3). The clinical records of 297 men (62 fertile with normozoospermia and 235 infertile men) were available. Of the 297 men, 27 (9%) reported inguinal hernia: 14 unilateral hernia, 5 bilateral hernia (one was a scrotal hernia) and 8 hernioplasty and orchiopexy (one with hydrocele). None of the 62 fertile men had a clinical history of varicocele, cryptorchidism or inguinal hernia. The number of men with inguinal hernia of the mutant groups was 5 out of 15 (33.3%) with 1090C > T mutation, 2 out of 4 (50%) with 1737G > A and none out of 7 with cluster mutation. Mutation 1090C > T was significantly associated with a history of inguinal hernia (P = 0.007) and the combination of cryptorchidism and inguinal hernia (P = 0.04). A significant association was also observed between the infrequent mutation 1737G > A and inguinal hernia (P = 0.043). No significant associations were found between the presence of USP26 mutations and varicocele or cryptorchidism.

**Discussion**

The present investigation of the five most frequently detected mutations in USP26 (370–371insACA, 494T > C, 1423C > T, 1090C > T and 1737G > A) (Paduch et al., 2005; Stouffs et al., 2005, 2006; Zhang et al., 2007) revealed that, although these mutations are not directly associated with male infertility, the 1090C > T mutation is associated (P = 0.007) with the presence of inguinal hernia.

The cluster of the three mutations and 1737G > A mutations apparently do not cause infertility since their presence in both fertile and infertile men do not make a significant difference. The segregation of the mutation in the two analyzed families (Fig. 2A and B) in which the propositus carry the mutations but their infertile brothers with similar fertility impairment do not, supports this contention. Furthermore, mutation 1737G > A was detected in normozoospermic fertile men. In addition, the amino acid positions of the cluster mutation were not evolutionarily well preserved and only position 475 may have altered the 3D structure of the USP26 protein domain (Fig. 1). Although mutation 1737G > A resulted in a
non-conservative amino acid substitution (methionine 579 tyrosine) at an evolutionarily preserved position and abolished a putative phosphorylation motif, it seems to be a rare polymorphism that may be associated with the appearance of inguinal hernia ($P = 0.043$). Its low frequency precludes making a clear-cut assessment of its role.

Our findings on the cluster mutation are in agreement with others (Ravel et al., 2006; Zhang et al., 2007) who reported compatibility between this mutation and being fertile, refuting Paduch et al.’s (2005) and Stouff et al.’s (2005) results. In addition, the greater frequency of this mutation among Israeli Arabs and Sephardic Jews than among Ashkenazi Jews fits the geographical frequency distribution reported earlier (Ravel et al., 2006; Stouff et al., 2006).

While case–control association studies are used to uncover the genetic basis of complex diseases, such study designs may lead to erroneous findings by population stratification (PS) (Gorroochurn et al., 2007). PS is a form of confounding that rises when cases and controls are sampled from genetically distinct populations or even when cases and controls are sampled from the same population but the latter is comprised of genetically distinct subpopulations. The current study meticulously subclassified the participants according to their mothers’ ethnic origins in order to prevent PS. The lack of ethnic classification of the study groups in previous studies may have led to the erroneous assumption that USP26 mutations may cause impaired spermatogenesis (Paduch et al., 2005; Stouffs et al., 2005). The absence of any correlation between familial infertility and the presence of the mutations undermine the idea of there being mutations with partial penetrance.

The fifth mutation (1090C $>$ T) is the one most frequently detected in the Israeli population. The frequency observed between infertile men in our study (4.7%) and that in Paduch et al.’s (2005) study (4.3%) is similar. Unlike their findings, however, we also detected the mutation among fertile men (3.7%), and the frequency difference between the fertile and infertile groups was not significant. This discordance may have resulted from the number of fertile men analyzed in each study (287 men in our study and 17 men in their study) and from a PS error (they failed to note ethnic origin). The highest mutant frequency was among Sephardic Jews, particularly the infertile ones. The detection of the mutation in a fertile brother (Fig. 2D), in two azoospermic men with normal spermatogenesis in testis and in two normozoospermic fertile men support our claim that mutation 1090C $>$ T itself does not necessarily affect fertility. However, the mutation may affect the protein function because it resulted in a non-conservative amino acid substitution (leucine 364 phenylalanine) at an evolutionarily well-preserved position.

Mutation 1090C $>$ T is highly associated ($P = 0.007$) with a previous inguinal hernia. The inguinal hernia prevalence among the men with 1090C $>$ T mutation (5 of 15 men, 33.33%) was found to be much higher than that reported in the general and infertile population, i.e. 5 and 6.65%, respectively (Yavetz et al., 1991). Since male infertility is more common among men with inguinal hernia and might be a common postoperative complication of hernioplasty as well, it is not surprising that the mutation is more frequently detected among infertile men.

An inguinal hernia represents a situation in which hernial contents are present in the inguinal canal, whereas a scrotal hernia refers to the presence of hernial contents in the scrotum. Inguinal and scrotal hernias may arise from a failure to achieve complete closure of the processus vaginalis, as well as a failure of involution at the internal inguinal ring. Several studies have suggested that genetic factors, such as INSL3, are involved in the development of inguinal and scrotal hernias (Cook et al., 2000; Kubota et al., 2002). A genome scan has revealed seven possible quantitative trait

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**Figure 1:** The impact of three mutations found in the USP domain on the USP26 three-dimensional structure. (A) Location of the relevant amino acids in the domain. (B) Close look at the amino acids changes. Blue and red denote normal and mutant structures, respectively. Yellow and green denote normal and mutant amino acid, respectively.
loci for inguinal and scrotal hernias in pigs (Grindflek et al., 2006). These regions included some promising candidate genes (INSL3, INSL5, MIS, CGRP, ESR1, COL9A1 and COL2A1). USP26 may be a new genetic factor involved in this impairment. The inguinocrotal phase of testicular descent is dependent on androgens. Viner et al. (1997) reported that 76% of men with complete androgen insensitivity syndrome (AIS) presented with an inguinal hernia, and that one-half of the complete AIS patients had an established family history of the disorder. It was recently reported in the Symposium on Ubiquitin and Signaling that mouse USP26 is a deubiquitinating enzyme for androgen receptor regulation (Kaiser and Fon, 2007).

The recently reported X-linked mental retardation syndrome may support the involvement of mutated USP26 in inguinal hernia (Cilliers et al., 2007). The syndrome includes primary testicular failure and late-onset testicular ascent. It maps to Xq25-q26. The telomeric boundary of the linkage is defined by marker DXS994 close (0.183 Mb) to the USP26 gene location. The tested marker that follows (DXS1062) is excluded from the linkage region and is located 5 Mb downstream from USP26. Although more than 70 genes are included in the syndrome’s locus, there is a possibility that USP26 impairment contributed to the late-onset testicular ascent reported in some of the family members (two out of the four patients and in one non-retarded child) (Cilliers et al., 2007).

The present investigation supports a lack of direct association between the USP26 mutations analyzed herein and infertility. Moreover, the presence of USP26 mutations are not associated with testosterone concentration below the normal range or with lower testicular volume as previously reported (Paduch et al., 2005). Nevertheless, the non-significant higher frequency of the USP26 mutations among infertile men might also suggest an involvement of the mutations in infertility when they are present with at least one other impairment factor. The co-factor hypothesis remains unproven to date. Such seems to be the relationship between partial AZFc deletions and impairment of spermatogenesis that was suggested to be associated with specific chromosome Y haplotypes (Yang et al., 2008).

In conclusion, mutant USP26 (1090C > T) may be an indirect cause of infertility by being a genetic risk factor for

### Table III. Frequency of USP26 mutations in the study groups and according to the maternal ethnic origin

<table>
<thead>
<tr>
<th>USP mutations</th>
<th>Total men (587) % (n)</th>
<th>Arabs (145) % (n)</th>
<th>Ashkenazi (187) % (n)</th>
<th>Sephardic (166) % (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fertile</td>
<td>Infertile</td>
<td>Fertile</td>
<td>Infertile</td>
</tr>
<tr>
<td>Clusterb</td>
<td>2.1 (6)</td>
<td>3 (9)</td>
<td>2.7 (2)</td>
<td>6.9 (5)</td>
</tr>
<tr>
<td>1090 C &gt; T (L364F)</td>
<td>3.5 (10)</td>
<td>4.7 (14)</td>
<td>1.4 (1)</td>
<td>4.2 (3)</td>
</tr>
<tr>
<td>1737 G &gt; A (M579I)</td>
<td>0.7 (2)</td>
<td>1 (3)</td>
<td>1.4 (1)</td>
<td>0</td>
</tr>
</tbody>
</table>

*There were 89 men with unknown ethnicity included in the total group calculations. There were four infertile men with unknown ethnicity who had USP26 mutations (two had L364F mutations and two had M579I mutations); b cluster includes mutations 370–371insACA (T123–124ins), 1423C > T (H475Y) and 494T > C (L165S) that were found in the same allele. n, number of men.

**Figure 2**: Pedigree of four families (A–D) with USP26 mutations. The black square denotes infertile men and the gray symbol denotes married ones without children. Rhombus pattern denotes sex unspecified. The number in the upper left, outside of the symbol, denotes the tested person. The number inside the symbol denotes the number of offspring of the indicated sex. Arrow: man with USP26 mutations. Double line: consanguineous marriage. Asterisk: persons with karyotype 46XY, inv(9)(p11;q13). Tent (^): persons with Familial Mediterranean Fever.

**Figure 3**: Frequency (%) of clinical findings. *Significant difference between the mutant and the total group. The number of men was 297, 7, 4 and 15 for the total, cluster, 1737 G > A and 1090C > T groups, respectively.

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developing inguinal hernia. Further assessment of the mutation in a large group of men with a history of inguinal hernia is needed to confirm our preliminary findings. We suggest that inguinal hernia might be included in the list of TDS-linked disorders. It will be of great interest to assess the correlation between the incidence of the USP26 mutation, inguinal hernia and TDS in countries like Denmark, which has a high frequency of TDS-related symptoms, compared with Finland, since both countries have well-documented registries. These kinds of studies are important to isolate genes involved in different sterility phenotypes and to identify causes of TDS obliteration.

**Supplementary data**

Supplementary data are available at HUMREP Journal online.

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