Expression patterns and transcript concentrations of the autosomal DAZL gene in testes of azoospermic men

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The DAZ (Deleted in AZoospermia) gene cluster on the Y chromosome is a strong candidate for the azoospermia factor. The DAZ gene was derived from an autosomal homologue, DAZL (DAZ-Like). This study was designed to assess the functional role of DAZL in human spermatogenesis. The expression patterns and mRNA transcript levels of DAZL in the testes of 17 azoospermic men were therefore examined by immunohistochemical staining and quantitative competitive reverse transcription–polymerase chain reaction. DAZL protein was expressed in the cytoplasm of primary spermatocytes and weakly in spermatogonia. It was detected in the testicular tissues of all subjects with germ cells present. The copy number of the DAZL transcript in normal spermatogenesis (n = 4), hypospermatogenesis or maturation arrest (n = 6), and Sertoli cell-only syndrome (n = 7) ranged from 1.22 × 10⁶ to 1.63 × 10⁶ per ng of RNA, 1.19 × 10⁵ to 2.82 × 10⁵ per ng of RNA and 2.83 × 10⁴ to 1.23 × 10⁵ per ng of RNA respectively. DAZL transcripts were lower in men with spermatogenic failure, and a significant difference was found between the three groups (P < 0.0001). This study suggests that DAZL may play an important role in the human spermatogenic processes of both mitosis and meiosis.

Key words: azoospermia/DAZL/spermatogenesis/testis

Introduction

Recent studies have shown that deletions of the azoospermic factor (AZF) region on the long arm of the Y chromosome (Yq) result in spermatogenic failure. These deletions occur in three non-overlapping subregions, named the AZFa, AZFb and AZFc regions (Reijo et al., 1995; Vogt et al., 1996). AZFc is the most commonly deleted subregion, and the DAZ (Deleted in AZoospermia) genes, which encode putative RNA-binding proteins, are strong AZFc candidate genes for defects in human spermatogenesis. They have been found to be completely deleted in 10–15% of azoospermic men (Shan et al., 1996; Simoni et al., 1998; McLreavey and Krausz, 1999). The DAZ gene family consists of a cluster of DAZ genes on the Y chromosome, and a single autosomal homologue, DAZL (DAZ-Like), on chromosome 3p24. The DAZL gene is highly homologous to the DAZ genes, with 83% similarity in the coding region of the cDNA (Saxena et al., 1996; Shan et al., 1996; Yen et al., 1996; Chai et al., 1997). Although frequent deletion of the DAZ genes suggests important roles for DAZ in spermatogenesis, the variable penetration of AZFc deletions that remove the entire DAZ gene cluster is also consistent with a certain degree of functional redundancy. An extreme view is that the Y-linked DAZ genes play a minor role in human spermatogenesis (Agulnik et al., 1998).

It is believed that the DAZ gene arose 40 million years ago, during primate evolution, from the transposition, repeat amplification, and pruning of an ancestral autosomal gene DAZL (Saxena et al., 1996). In addition to human beings, DAZ orthologues are present only on the Y chromosomes of great apes and Old World monkeys. Other mammals have only the autosomal Dazl gene (Cooke et al., 1996; Reijo et al., 1996; Saxena et al., 1996; Shan et al., 1996; Yen et al., 1996; Seboun et al., 1997). In many species, Dazl homologues are essential for the differentiation of germ cells. For example, the loss of boule results in a meiotic arrest and azoospermia in Drosophila (Eberhart et al., 1996). A loss of germ cells and absence of gamete production has been observed in Dazl knockout mice (Ruggiu et al., 1997). When a human DAZ transgene is introduced into Dazl-null mice, a partial rescue of the mutant phenotype is observed (Slee et al., 1999). These findings suggest a high degree of functional conservation between the DAZ and DAZL genes. There are, however, no reported
instances of DAZL gene mutations in infertile men. Whether DAZL plays a crucial role in spermatogenesis in humans also merits investigation. Given this evidence of functional redundancy for DAZ, it would be tempting to postulate that DAZL and DAZ operate in a complementary or synergistic manner during human spermatogenesis.

One approach to assessing the functional role of DAZL in human spermatogenesis is to test its expression patterns and levels in the testes. In the present study, testicular tissues were obtained from azoospermic men with normal spermatogenesis (obstructive azoospermia) or varied degrees of spermatogenic failure (non-obstructive azoospermia). The expression patterns of DAZL were examined with a polyclonal antibody to DAZL and immunohistochemical staining. The levels of DAZL transcripts were measured by quantitative competitive reverse transcription–polymerase chain reaction (QC-RT–PCR). To the best of our knowledge, this is the first report on quantifying the amount of DAZL RNA transcripts in human testicular tissue.

Materials and methods

Patients

Seventeen patients with azoospermia were included in this study. The patients agreed to provide an extra-piece of testicular specimen for immunohistochemical study and QC-RT–PCR during their testicular biopsy or sperm retrieval procedures. Informed consent was obtained from all patients. They underwent a comprehensive examination, biopsy or sperm retrieval procedures. Informed consent was obtained from all patients. They underwent a comprehensive examination, including a detailed history taking, physical examination, endocrinology profile testing (LH, FSH, prolactin, and testosterone), chromosome analysis, and Y chromosome microdeletion test. In patients with congenital bilateral absence of the vas deferens, which was diagnosed by physical examination or scrotal exploration, no further examination was done. The remaining patients underwent transrectal ultrasound, vaso-vasculography, and bilateral testicular biopsies, and the aetiologies of azoospermia were determined accordingly.

Total RNA isolation and cDNA synthesis

The testicular tissues from patients were examined. Ovarian tissue was taken from women between 18 and 48 years old who underwent oophorectomy for benign conditions. The following types of tissue were also collected from a 20 week male abortus: liver, spleen, lung, kidney, testis, skin and placenta. The tissues were stored in liquid nitrogen using 2-methylbutane as cryoprotectant until use. Before isolation of total cellular RNA, each specimen was sliced into 10 µm thick pieces. Total cellular RNA was extracted using standard methods (High Pure™ RNA Tissue Kit; Boehringer Mannheim, Indianapolis, IN, USA), and quantified by total absorbance at 260 nm. For the synthesis of complementary DNA (cDNA), 12 µl aliquots of master mixture containing 2 µl of RNA, 1 µl of oligo(dT)$_{12-18}$ primer (500 ng/µl) (Gibco/BRL, Grand Island, NY, USA), and 9 µl of DEPC-treated water were heated to 70°C for 10 min and put on ice. RT reactions were performed in 20 µl aliquots containing master mixture, 4 µl of 5× first strand synthesis buffer, 0.1 mol/l dithiothreitol, 10 mmol/l of each dNTP, and 200 U of Superscript™ II RNase H$^-$ reverse transcriptase (Gibco/BRL). The RT temperature profile was 42°C for 1 h, 75°C for 15 min, and final cooling to 4°C. The cDNA was aliquoted and stored at −20°C until use.

Preparation of native and competitor RNA

The synthesis of native and competitor RNA templates for internal standards was performed according to the method described previously (Celi et al., 1993). For semi-quantitative RT–PCR, the housekeeping gene glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was also used as an internal control. Sequences for DAZL and GAPDH primers are shown in Table I. DNA sequences of the forward primer for the DAZL gene are completely absent in the DAZ gene. The terminal three nucleotides at the 3’ end of the reverse primer also completely mismatch with corresponding sequences of the DAZ gene. The design assures specificity of primers for the DAZL gene. In the present investigation, the DAZL amplicon is a 313 bp fragment that spans from exon 8 to the 3’-untranslated region. The size of DAZL competitor is 290 bp. The sizes of GAPDH amplicon were 679 and 552 bp for native control and competitor respectively. For the synthesis of internal standards and internal control, RNA extracted from the testicular tissue with normal spermatogenesis was subjected to RT followed by PCR. PCR amplifications were performed in 20 µl reactions containing 0.5 µmol/l of each primer, 1.5 mmol/l of MgCl$_2$, 200 µmol/l of each dNTP, 5 µl of cDNA, 1×PCR buffer, and 0.5 U of Taq polymerase (Gibco/BRL). The temperature profile was 30 cycles of amplification (94°C for 1 min, 60°C for 90 s, and 72°C for 1 min), and a final extension at 72°C for 5 min. The PCR products were separated on a polyacrylamide gel with 1×TBE (90 mmol/l Tris, 90 mmol/l boric acid, 1 mmol/l EDTA, pH 8) buffer at 110 V for 30–50 min. After initial confirmation by gel electrophoresis, PCR products of competitor and native control of DAZL and GAPDH genes were purified using the Concert™ Rapid PCR Purification System (Gibco/BRL) and subcloned into a pT7Blue T-vector (Novagen, Madison, WI, USA). The inserts were confirmed by sequencing using an automatic sequencer (ABI 377; Applied Biosystems/PE, Foster City, CA, USA).

Quantification of DAZL mRNA transcripts by QC-RT–PCR

Quantification by QC-RT-PCR has been described elsewhere (Tsai and Wiltbank, 1996). Briefly, competitor and native RNA were synthesized by in-vitro transcription (Riboprobe In Vitro Transcription System; Promega, Madison, WI, USA). The RNA product was extracted and quantified by total absorbance at 260 nm. RT was performed using 10 fmol of in-vitro transcribed RNA. The QC-RT–PCR assay consisted of 2 amol of competitor RNA and serial dilutions of native RNA (from 0.2 to 25.6 amol) or RNA from unknown samples in a final volume of 20 µl of RT mixture. The RNA was

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer</th>
<th>Sequences</th>
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<tr>
<td>DAZL</td>
<td>Forward</td>
<td>5’-GGAGCTTATGTTGTAACCTCC-3’</td>
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<tr>
<td></td>
<td>Reverse</td>
<td>5’-CCATTGTAACCTAAGTAAGCCAG-3’</td>
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<tr>
<td></td>
<td>Reverse (competitor)</td>
<td>5’-CCATTGTAACCTAAGTAAGCCAG-3’</td>
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<tr>
<td>GAPDH</td>
<td>Forward</td>
<td>5’-TGCCGCTTTCACACCACAT-3’</td>
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<tr>
<td></td>
<td>Reverse</td>
<td>5’-CCACCACCTTCTTCTGTA-3’</td>
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<tr>
<td></td>
<td>Reverse (competitor)</td>
<td>5’-CCACCACCTTCTTCTGTA-3’</td>
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added to an RT master mix (50 mmol/l Tris–HCl, 75 mmol/l KCl, 3 mmol/l MgCl₂, pH 8.3, 10 mmol/dithiothreitol, 100 pmol random primer, 4 mmol/l dNTP and 50 IU M-MLV reverse transcriptase). RT was performed at 42°C for 60 min followed by heating to 95°C for 10 min and quick-chilled to 4°C in a programmable thermocycler (PTC-100; MJ Research, Watertown, MA, USA). Five µl of RT products was added to 15 µl of PCR mix (final concentration: 20 mmol/l Tris–HCl, 50 mmol/l KCl, 1.5 mmol/l MgCl₂, 0.2 mmol/l dNTP, 0.5 IU Taq polymerase, and 0.4 mmol/l of primers). This was subjected to 30 cycles of amplification (30 s denaturation at 95°C, 30 s annealing at 57°C, and 30 s elongation at 72°C) followed by final elongation at 72°C for 5 min. The PCR products were separated on a polyacrylamide gel (5% for GAPDH, 8% for DAZL), stained with ethidium bromide, and analysed by alpha-image (Alpha Innotech, San Leandro, CA, USA). A ratio was calculated for the intensity of native versus competitor bands on each lane of the gels. To produce the standard curve, the logarithmic ratio of native to competitor was plotted against the logarithmic initial amounts of native. Concentrations of specific transcripts were calculated by interpolation as previously described (Tsai and Wiltbank, 1996, 1997).

**Generation of anti-DAZL antibody and Western blotting**

The COOH terminal of the DAZL protein is completely different from that of the DAZ protein. A peptide consisting of amino acid residues 272 to 290 (TQDDYFKD KRV HFRRSRA) in the COOH terminal of the DAZL protein (GenBank accession No. NP_001342) was synthesized (Sigma-Genosys, Woodlands, TX, USA). It was conjugated to a carrier protein (Imject Maleimide Activated mcKLH was synthesized (Sigma-Genosys, Woodlands, TX, USA). It was terminal of the DAZL protein (GenBank accession No. NP_001342) from that of the DAZ protein. A peptide consisting of amino acid sequences 120-140 (QIEEYEW) of the DAZL protein (GenBank accession No. NP_001342) was tested by Western blot when the titre was 12 000. Serum antibodies were then affinity-purified on protein A columns according to the manufacturer’s instructions (Immunopure® Plus Immobilized Protein A IgG Purification Kit; Pierce). For Western blot analysis, the various tissues were homogenized and mixed with twice the volume of lysis buffer containing protease inhibitor (T-PER® Tissue Protein Extraction Reagent; Pierce). After complete mixing, the samples were put on ice for 30 min and sonicated vigorously for 20 s to shear chromosomal DNA. Approximately 20 µg of total proteins were then fractionated on a 12% sodium dodecyl sulphate–polyacrylamide gel, and transferred to PVDF membranes (Millipore, Bedford, MA, USA) using a BioRad transfer system at 110 V for 1 h. The membranes were washed with Tris-buffered saline (TBS) containing 0.5% of Tween 20, and then incubated in a blocking solution (5% solution of mild powder in the wash buffer) for 1 h. The membranes were then incubated in a 1:100 to 1:250 dilution of a primary antisera overnight and washed three times with the wash buffer, followed by incubation with a 1:10 000 dilution of the secondary antibody (goat anti-rabbit IgG, peroxidase-conjugated; Pierce) in the wash buffer. The filters were then washed several times, and the peroxidase activities were visualized using the SuperSignal substrate according to the manufacturer’s instructions (Pierce).

**Immunohistochemistry study**

Bouin’s fixed human testicular specimens were dehydrated, embedded in paraffin, and sectioned at 5 µm. The sections were deparaffinized with 100% xylene and sequentially rehydrated with 100, 95 and 70% ethanol. The slides were then blocked with 3% hydrogen peroxide in absolute methanol for 5 min, washed with water for 5 min, and heated at 90°C for 5 min in pre-heated citrate buffer. After cooling, the slides were washed twice with TBS for 5 min each time. The slides were incubated with the primary antibody (1:1000) for 60 min at room temperature. Following the washing steps with TBS, sections were incubated with biotinylated mouse anti-rabbit IgG antibody (Dako, CA, USA) for 30 min at room temperature, washed with TBS, then incubated with the avidin–biotin complex for 30 min at room temperature, followed by reaction with DAB (diaminobenzidin tetra-chloride)/hydrogen peroxide. Sections were subsequently counterstained with haematoxylin, dehydrated, and mounted. The specificity of the antibody against DAZL protein was examined using pre-immune rabbit serum as a negative control.

**Results**

**Patients**

Among the 17 patients with histological information, four were diagnosed with irreparable obstructive azoospermia and normal spermatogenesis. Non-obstructive azoospermia was diagnosed in the remaining 13 patients, and their histopathological findings were hypospermatogenesis (four patients), maturation arrest (two patients), and Sertoli cell-only syndrome (seven patients). All 17 patients had a normal male karyotype. One patient with hypospermatogenesis was found to have an AZFc microdeletion encompassing the DAZ gene cluster (markers deleted: sY277, sY283).

**Validation of standard curve and quantification of DAZL transcripts in the testicular tissues**

QC-RT–PCR was used to measure the amount of DAZL transcripts in biopsied testicular tissue. The logarithmic ratio of native to competitor was plotted against the logarithmic amount of native to produce the standard curve. The standard curve was highly reproducible (data not shown) and the R² values of the standard curve for GAPDH and DAZL were 0.9987 and 0.9984, respectively (Figure 1). The specificity of primers for the DAZL gene was further tested with a clone containing DAZL cDNA (provided by Dr Pauline Yen, Harbor-UCLA Medical Center, USA) and various tissues including liver, spleen, lung, kidney, testis, skin and placenta. We were able to detect DAZL transcripts only in testicular tissues including a Sertoli cell-only syndrome specimen (Figure 2). The ratio of products for unknown testicular samples was logarithmically transformed and interpolated to calculate the absolute amount of transcripts. In four men with normal spermatogenesis, the GAPDH and DAZL copy numbers ranged from 8 × 10³ to 2.06 × 10⁴ and from 1.22 × 10⁶ to 1.63 × 10⁶ per ng of RNA respectively. In six men with hypospermatogenesis or maturation arrest, the GAPDH and DAZL copy numbers ranged from 1.05 × 10⁴ to 7.34 × 10⁴ and from 1.19 × 10⁵ to 2.82 × 10⁵ per ng of RNA respectively. In seven men with Sertoli cell-only syndrome, the GAPDH and DAZL copy numbers ranged from 9.7 × 10³ to 2.83 × 10⁴ and from 2.83 × 10⁴ to 1.23 × 10⁵ per ng of RNA, respectively. The Kruskal–Wallis test showed no difference (P = 0.093) between the GAPDH RNA copy in the three azoospermic groups, but a significant difference in DAZL RNA copy numbers was noted between
Figure 1. Standard curve of GAPDH gene and DAZL gene quantitative competitive reverse transcription–polymerase chain reaction (QC-RT–PCR). (a) The bands for 0.5–64 amol of native (GAPDH) co-amplified with 2 amol of competitor (GAPDH-127) for 30 cycles and separated on a 5% polyacrylamide gel. (b) The bands for 0.2–25.6 amol of native (DAZL) co-amplified with 2 amol of competitor (DAZL-44) for 30 cycles and separated on a 8% polyacrylamide gel. The log ratio of native to competitor product was plotted against the log amount of initial native added to the RT–PCR.

Figure 2. (a) Constant copy number of GAPDH transcripts in different tissues. The expected sizes of the polymerase chain reaction (PCR) products were 679 bp for native and 552 bp for competitor GAPDH. (b) Presence of DAZL transcripts exclusively in the testicular tissues. The expected sizes of the PCR products were 313 bp for native and 290 bp for competitor DAZL. SCO = Sertoli cell-only; NC = negative control.

Immunoblotting of testis proteins

The presence of DAZL in the human testis, ovary, and other tissues were examined by Western blot analysis. Using a pre-immune serum, we were unable to detect the protein band in any type of tissue. Western blots with anti-DAZL revealed a single band representing a protein of ~33.5 kDa, exclusively in testis extract (Figure 4).

Immunohistochemistry study

Immunohistochemical analysis was performed to determine the localization of DAZL protein in different testicular samples. The DAZL protein was seen most abundantly in the cytoplasm of primary spermatocytes and weakly in the cytoplasm of spermatagonia. These findings were consistent in testicular specimens from patients with normal spermatogenesis, hypospermatogenesis, and maturation arrest; however, DAZL protein was not detected in the testes of patients with Sertoli cell-only syndrome. No signals were observed when the testicular specimens were stained with pre-immune serum (Figure 5).

Discussion

Competitive RT–PCR has been used to quantify the expression of multiple gene products from limited amounts of tissue (Porcher et al., 1992). Our protocol produces one standard curve with different amounts of standard RNA amplified with a constant amount of competitor RNA (Tsai and Wiltbank, 1996). Nevertheless, the conventional competitive ‘equimolar’ RT–PCR methods use serial dilutions of competitor with a constant amount of unknown RNA in the reaction (Porcher et al., 1992). Compared with the conventional equimolar RT–PCR protocol, our method requires minimal amounts of RNA because the multiple reactions at different concentrations of competitor RNA are not necessary. In addition, our protocol does not require that the amplification efficiency of the native and competitor be equivalent (Tsai and Wiltbank, 1996). Unlike conventional protocols for PCR quantification, our method allows the reaction to reach a plateau, thus maximizing the sensitivity and facilitating detection of rare transcripts with a minimal amount of sample. Recently, real-time PCR has become a convenient tool for measuring amounts of gene expression (Heid et al., 1996). However, the equipment is too
DAZL gene expression in human testes

**Figure 3.** (a) GAPDH copy number of RNA in different testicular phenotypes. No significant difference was noted between the three groups ($P > 0.05$, Kruskal–Wallis test and Dunn’s tests for posterior comparison). (b) DAZL copy number of RNA in different testicular phenotypes. A significant difference was noted between the three groups ($P < 0.0001$, Kruskal–Wallis test). Pairwise comparisons of DAZL copy number in the three groups showed a significant difference between the NR and SCO groups ($P < 0.01$, Dunn’s test for posterior comparison). NR = normal spermatogenesis; HS+MA = hypospermatogenesis + maturation arrest; SCO = Sertoli cell-only.

**Figure 4.** Western blot analysis of DAZL protein. A protein of ~33.5 kDa was detected exclusively in human adult testis. expensive to be afforded by every laboratory. Other methods that could be used to measure gene expression include serial analysis of gene expression (SAGE) and microarray (Schena et al., 1995; Velculescu et al., 1995); however, both methods require relatively large amounts of tissue. We believe that our QC-RT–PCR protocol provides maximum sensitivity and can be used to evaluate gene expression from very limited amounts of experimental material, such as a sample from a testicular biopsy.

In this study, significantly higher DAZL mRNA concentrations were found in azoospermic men with normal spermatogenesis than in those with non-obstructive azoospermia. It is not surprising that the level of DAZL transcripts is lower in testes with spermatogenic defects, because DAZL is expressed in primary spermatocytes and spermatogonia. A lower level of transcripts may be attributed to the presence of fewer germ cells. In this series, however, DAZL transcripts were expressed in small amounts in specimens from Sertoli cell-only syndrome patients, in contrast with the finding of immunohistochemical analysis, which detected no DAZL protein in testicular specimens from these patients. The discrepancy could be explained by the following hypotheses: (i) DAZL is expressed in the nucleus or cytoplasm of some types of non-germ cells. The protein level is difficult or impossible to detect with immunostaining because of their nucleus location or the low expression level in these cells. The DAZL gene has been shown to be expressed in female granulosa cells of primordial follicles (Brekhman et al., 2000). With respect to sexual differentiation in mammals, both Sertoli cells in the testis and granulosa cells in the ovary are derived from the coelomic epithelium of the genital ridge (George and Wilson, 1992). Therefore, it is plausible to postulate that DAZL is also expressed in the non-germ lineage of the testis, such as Sertoli cells. (ii) Inadequate formation of DAZL protein may be due to post-transcriptional gene silencing, RNA degradation, post-translational processing, or protein degradation (Grant, 1999; Wickner et al., 1999). (iii) The transcripts detected by RT–PCR represent illegitimate transcription of the DAZL gene in somatic cells, such as endothelial cells, fibroblastic cells, or myeloid cells, and are not functionally significant (Chelly et al., 1988, 1989). This possibility can probably be excluded considering the absence of DAZL transcripts in various tissues tested other than testicular and ovarian tissues. (iv) Sertoli cell-only specimens actually contain some foci with germ cells. There have been reports of men with non-obstructive azoospermia who have undergone testicular sperm extraction for intracytoplasmic sperm injection (Tournaye et al., 1997). (v) The possibility of sample contamination cannot be ruled out. Nevertheless, we believe that presence of DAZL transcripts is not due to tissue contamination because DAZL transcripts were invariably detected in all seven Sertoli cell-only specimens. DAZL transcripts have also been found in six of 20 men with Sertoli cell-only syndrome undergoing testicular sperm extraction, including one with a wet preparation showing no spermatogenic
Figure 5. Immunohistochemical localization of DAZL protein in human testes with different phenotypes. No staining was observed in tissue sections from pre-incubation with pre-immune antisera (a). DAZL staining was seen in tissue sections with normal spermatogenesis (b, c), maturation arrest (d), and hypospermatogenesis (e), whereas a tissue section from Sertoli cell-only syndrome was negative (f). Note that strong DAZL expression was detected in the cytoplasm of primary pachytene spermatocytes (arrowheads) and a weaker but clearly detectable signal was present in spermatogonia (arrows). Original magnification of a, b, d–f, ×400; scale bars = 200 µm. Original magnification of c, ×1000; scale bar = 80 µm.

cells (Lee et al., 1998). Further studies are required to solve the discrepancies between RT–PCR results and immunohistochemical staining.

Some conflicts have been found in the literature concerning the expression pattern of DAZL transcripts in mammals. By analysing the mouse Dazl expression pattern by RT–PCR, it has been stated (Cooke et al., 1996) that peak expression is detected 20 days after birth, indicating maximal expression in pachytene spermatocytes or round spermatids. However, another study (Reijo et al., 1996) found that peak expression occurs between postnatal days 6 and 10, indicating maximal expression in spermatogonial stem cells. In addition, the expression pattern of Dazl mRNA in mouse testes examined by in-situ hybridization (Niederberger et al., 1997) showed that the expression was highly restricted to pre-meiotic stages IV–VI of spermatogenesis. Conflicts also exist in the reported protein levels of DAZL. Using immunohistochemical analysis in adult rat testes (Rocchietti-March et al., 2000), a strong expression of Dazl protein in the cytoplasm of primary pachytene spermatocytes, and a weak expression in spermat-
gonia, early spermatocytes, and elongated spermatids were observed. Another report (Ruggiu et al., 1997), showed that Dazl protein was only detectable in type-B spermatogonia and primary spermatocytes of the adult mouse. In a study on human beings, DAZL protein was shown to be present in male germ cells in many stages during spermatogenesis and spermiogenesis (Reijo et al., 2000), and the nuclear localization of DAZL protein was also observed in gonocytes and spermatogonia. However, another study has demonstrated that DAZL is expressed in a dynamic way during human spermatogenesis, with DAZL protein being located in the nuclei of gonocytes, and relocated to the cytoplasm in adults (Ruggiu et al., 2000). In the present study, we did not observe nuclear localization of DAZL protein with either immunohistochemical staining or immunofluorescence staining (data for immunofluorescence staining not shown); nor could DAZL protein be detected in post-meiotic spermatids. Because antibodies from different groups were raised against different parts of DAZL protein, it is possible that different epitopes have different presentation for different cell types. It is also possible that expression patterns of DAZL transcripts or protein are different among different mammalian species. Nevertheless, taken together with the findings of all published investigations, it can be postulated that the autosomal DAZL gene plays a distinct role in the regulation of human spermatogenesis.

In species other than Old World monkeys and human beings, a single copy of the DAZL gene is sufficient to maintain spermatogenesis in many species, such as Dazl in the mouse and boule in Drosophila. The reasons for the additional requirement of a Y-chromosome DAZ gene family in humans are still unclear. During evolution, the DAZ gene cluster may give a quantitative dosage effect to provide a reproductive advantage (Slee et al., 1999). In the present study, one patient with hypospermatogenesis and complete deletion of Y-chromosome DAZ gene cluster family showed the lowest copy number of DAZL transcripts in the group of hypospermatogenesis and maturation arrest. Obviously, no conclusion could be drawn with a very limited sample size. Questions of whether DAZ and DAZL genes are functionally complementary or synergistic, and whether either one of them is functionally redundant, remain to be elucidated.

It is believed that many genes are involved in male infertility, and defects in these genes may result in oligozoospermia or azoospermia. It is possible that a defective autosomal DAZL gene may be responsible for spermatogenic defects in some cases and that its genetic defect is likely to be inherited in an autosomal recessive fashion. It has been stated (Lilford et al., 1994) that infertility could be inherited in an autosomal recessive mode in a subset of patients presenting with male infertility. In order to explore the role of DAZL in male infertility inherited in an autosomal recessive pattern, large-scale mutation analysis and linkage studies are needed.

In this study, we have shown the expression pattern and transcript level of the DAZL gene in human testes with normal spermatogenesis and spermatogenic defects. The level of DAZL transcripts was markedly lower in men with hypospermatogenesis, maturation arrest, and Sertoli cell-only syndrome. Immunohistochemical staining revealed that DAZL expression was found predominately in the cytoplasm of primary spermatocytes and weakly in spermatogonia. These data suggest that DAZL may be involved in mitosis and meiosis in the human testes.

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