Lack of the T54A polymorphism of the DAZL gene in infertile Italian patients

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The Thr54Ala polymorphism of the deleted-in-azoospermia-like (DAZL) protein has been associated with susceptibility to spermatogenic failure in the Taiwanese population. We used single-strand conformation polymorphism and restriction fragment analyses to investigate the presence of the A → G transition in exon 3 of the DAZL gene in 95 infertile Italian patients. The patients had oligozoospermia or non-obstructive azoospermia with different degrees of testicular cytological picture. The allele carrying T54A polymorphism was not present in this group of patients nor in 63 controls, indicating that the frequency of this putative mutation is <1% in Italy. Since the Italian population usually shows allelic frequencies similar to the other Caucasian populations, we suggest that the T54A allele might play a role in infertility only in Taiwanese or Asiatic individuals.

Key words: azoospermia/DAZL/male infertility/oligozoospermia

Introduction

In Italy, as in the other Caucasian populations, 10–15% of couples are infertile, and a male factor is present in half of the cases (Foresta et al., 2002). Male infertility has a genetic cause in 10–15% of patients. Since spermatogenesis is a complicated multistep process, and failure in any of these steps can lead to oligozoospermia or azoospermia, there is not a unique genetic cause of male infertility (Foresta et al., 2002).

The frequencies of known mutations in the Italian population correspond to the average frequencies in the Caucasian population. Yq microdeletions are detected in 5–15% of males with spermatogenic failure, prevalence increasing with severity of testicular damage (Foresta et al., 2001). Among these cases, deletion involving the DAZ (deleted in azoospermia) gene family are the most frequent (3–15% in azoospermia, 5% in severe oligozoospermia) (Foresta et al., 2001).

DAZL (DAZ-like), on chromosome 3p24, is the autosomal homologue of the DAZ gene. The four copies of the DAZ gene on chromosome Y are identical to each other, while the DAZL gene shares 83% similarity in the coding region. The product of these two genes are RNA-binding proteins expressed only in testis (DAZ) or testis and ovary (DAZL) with still unknown functions (Foresta et al., 2001). It is likely that the DAZ gene arose from the transposition, repeat amplification, and pruning of DAZL (Saxena et al., 1996). These rearrangements happened late in evolution since the chromosome Y DAZ gene is present only in humans, great apes and Old World monkeys. Mice, like other mammals, have only the autosomal Dazl gene. Dazl knockout mice have loss of germ cells though absence of gametes. A human DAZ transgene confers partial rescue of the mouse Dazl null sterility (Slee et al., 1999), showing the functional conservation of DAZ and Dazl.

Even if DAZL seems to play a crucial role in male fertility, to date there are no mutations reported in infertile men (Van Golde et al., 2001). A single-nucleotide polymorphism (SNP) at nucleotide 386 of the coding region of DAZL cDNA leading to Thr54 → Ala change (T54A) in the RNA recognition motif has been reported to be more frequent in a group of Taiwanese patients with severe oligozoospermia and non-obstructive azoospermia than in controls (7.39 versus 0.86%) (Teng et al., 2002). In order to evaluate if this SNP might also be associated with susceptibility to spermatogenic failure in the Italian population, we studied 95 infertile patients and 63 fertile controls.

Materials and methods

Subjects

We selected 95 patients referred to our Centre for fertility evaluation and affected by oligozoospermia or non-obstructive azoospermia. Semen analysis was performed according to the standard methods outlined by the World Health Organization (1999). Among them, 28 showed azoospermia, 51 severe oligozoospermia (sperm count <5 × 10⁶/ml), and 16 moderate oligozoospermia (sperm count 5–20 × 10⁶/ml). Twenty-four of the 95 patients (12 with azoospermia and 12 with severe oligozoospermia) had a history of unilateral or bilateral cryptorchidism, whereas the remaining 71 were classified as idiopathic infertile men.

All patients underwent physical examination, semen analyses, plasma determination of FSH, LH and testosterone, karyotyping, and molecular tests for Y-chromosome microdeletions (Foresta et al., 1997; Ferlin et al., 2003).

Patients with azoospermia and severe oligozoospermia underwent testicular fine needle aspiration cytology (Foresta et al., 1992) to relate the seminiferous tubules to testicular alteration. In particular, patients affected by azoospermia showed a testicular cytological picture of Sertoli cell-only syndrome (absence of germ cells in both testes), and patients with severe oligozoospermia had a picture of severe hypospermatogenesis (strong reduction of germ cells, without maturation arrestes).

Sixty-three fertile, normozoospermic men were enrolled as controls. Both the patients and the controls were Caucasians of Italian origin.
**PCR amplification**

Genomic DNA was extracted from peripheral blood samples using a mammalian blood isolation kit (Roche Diagnostics Corp., USA).

PCR were performed in 25 µl final volume reaction mix, containing 200 ng genomic DNA, 200 µmol/l dNTP, 100 ng of each primer, 8% dimethysulphoxide, and 0.1 IU Taq DNA polymerase (Experteam, Italy). The primers of DAZL exon 3 (DAZL3-F GAATGCTGAATTTTACTCTTTGAAG and DAZL3-R CTCTATACGTGGCTAGATTC) give an amplification product of 181 bp (Teng et al., 2002).

The PCR cycles (94°C for 1 min, 58°C for 1 min, 72°C for 1 min repeated 37 times) were performed in a PCRexpress thermal cycler (Hybaid Ltd, UK).

**Mutation screening by single-strand conformation polymorphism (SSCP)**

Two microlitres of each PCR product was mixed with 4 µl of denaturation buffer (95% formamide, 10 mmol/l EDTA, 0.1% Bromophenol Blue, 0.1% xylene cyanol). The mix was denatured for 6 min at 94°C and put on ice for ≥1 min.

Each denatured sample was subjected to SSCP analysis using GeneGel Excel 12.5% acrylamide gels as recommended by the manufacturer (Pharmacia Biotech, Sweden).

After SSCP analysis, he PCR products with an aberrant band were directly sequenced with an automatic sequencer (ABI Prism 3100; Applied, USA).

**Mutation detection by restriction enzymes in genomic DNA**

PCR products of exon 3 were digested using the restriction enzyme Alu I (New England Biolabs, USA). The restriction fragments were run on a polyacrylamide gel. The normal allele is cut into two restriction fragments of 66 and 115 bp, whereas the polymorphism A → G creates an Alu I restriction site (AGCT) giving three fragments of 53, 13 and 115 bp.

**In vitro creation of T54A positive control**

In order to have a T54A positive control, we PCR amplified a normal genomic DNA with primer DAZL3-F GAATGCTGAATTTTACTCTTTGAAG and a reverse primer overlapping the mutation site and containing the G instead of the A (AGCAGAAGCTTCAATCTCAAGCTTCA). This PCR product and a normal exon 3 amplicon were digested with Hind III (Roche, Germany) and run on a 10% acrylamide gel. The bands corresponding to the 5' of the mutated amplicon, and to the 3' of the normal exon 3 PCR product, were cut and the DNA fragments were extracted from the gel. The two pieces were ligated, PCR-amplified with DAZL3-F and DAZL3-R and sequenced. Once the sequence showed that the new PCR product had the T54A mutation, we used it as a positive control both alone to mimic the mutated homozygote, and mixed with normal PCR product to mimic the heterozygote. Figure 1 shows the mutated chromatogram and the SSCP patterns of these products (B).

**Results**

None of the exon 3 PCR products of 95 patients and 63 controls showed changes in electrophoretic mobility in polyacrylamide gels. Since the alteration in conformation might be detected as a change in intensity of bands, we directly sequenced three samples with stronger bands and one normal sample. In the four samples, sequences with forward primer gave a strong reproducible background leading to a putative A → G transition at nucleotide 386 in exon 3 (Figure 2A). The polymorphism was not present if the sequence was performed with the reverse primer (Figure 2B).

Comparable results were obtained digesting patients and controls with the Alu I restriction enzyme: all the restriction fragments were of the normal allele sizes. No additional bands were detected.

**Discussion**

Teng et al. (2002) have described a polymorphism of the DAZL gene, 386A → G, leading to the amino acid change T → A at position 54 of the DAZL protein. In the Taiwanese population, the T54A polymorphism in exon 3 is more prevalent in patients with spermatogenenic failure, suggesting that it may have a role in determining severe male infertility.

In the present study, we screened for the DAZL gene T54A variant 95 infertile patients and 63 controls in order to study the frequency of this SNP in the Italian population.

We did not find T54A alleles in the patient population, nor in the control population. The frequency of the T54A allele in the Italian population is therefore <1/316. Since the Italian population does not differ greatly from the general Caucasian population, we can suppose that the T54A variant is not largely present in the Caucasian population.

We can then assume that the T54A polymorphism is not one of the major causes of male infertility in the Western population.

The T54A variant might be a polymorphism characterizing the Taiwanese population, or one of its subgroups. In order to assess the real role played by this variant in male infertility, it is necessary to evaluate its presence in other populations and to compare the genetic origin of patients and controls of the Taiwanese study. It is possible that this polymorphism is present only in one of the five counties of
Southern Taiwan, indicating a very recent A → G transition. We did not analyse the entire DAZL gene, because it has been well studied by other groups and it was never found to be mutated in infertile males (Van Golde et al., 2001; Teng et al., 2002). Although DAZL is theoretically a candidate gene for male infertility given its homology to DAZ, its actual role in spermatogenesis remains to be elucidated.

References


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