A novel missense mutation P1290S at exon-20 of the CFTR gene in a Portuguese patient with congenital bilateral absence of the vas deferens

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Objective: To report a novel cystic fibrosis transmembrane conductance regulator (CFTR) gene missense mutation in a compound heterozygote with congenital bilateral absence of the vas deferens (CBAVD).

Design: Descriptive, controlled study.

Setting: Tertiary academic hospital genetics laboratory and private in vitro fertilization (IVF) clinic.

Patient(s): One 46-year-old man with CBAVD and no clinical cystic fibrosis (CF) phenotype as indicated by the advanced age at diagnosis, absence of chronic airways and gastrointestinal disease, and normal pancreatic function and sweat chloride concentration. Genomic blood DNA from the patient’s parents was analyzed to perform family studies, and 109 fertile men, 32 patients with CBAVD, 15 children carriers of one CFTR mutation, and 5 patients with CF were used to rule out polymorphism.

Intervention(s): Clinical evaluation and treatment, genetical screenings.

Main Outcome Measure(s): Clinical data, biochemical assays, spermogram analysis, testicle biopsy, intracytoplasmic sperm injection (ICSI) outcome, and CFTR whole gene mutation screening and IVS8T polymorphism.

Result(s): The DNA analysis revealed a 7T/7T homozygote at IVS8-T, with a 4000C→T change (P1290S) in exon 20 of the CFTR gene, which was inherited from the patient’s father. It was associated with a 3272-26A→G mutation in the other allele that was inherited from his mother.

Conclusion(s): The novel P1290S missense CFTR mutation causes an amino acid change in a highly conserved region of the CFTR protein that controls channel opening. Pathogenicity is suggested by development of CBAVD in association with a mild CFTR mutation. (Fertil Steril 2005;83:448–51. ©2005 by American Society for Reproductive Medicine.)

Key Words: CBAVD, CFTR, DGGE, IVS8-T, cystic fibrosis, male infertility

Cystic fibrosis (CF) is the most common autosomal recessive disease in the Caucasian population, with an incidence of 1 in 2500 live births and a carrier frequency of 1:25. It is caused by mutations in the CF transmembrane conductance regulator (CFTR) gene located on 7q31-q32, which comprises about 250 Kb, encompasses 27 exons, and encodes a transmembrane protein that forms a chloride channel regulated by adenosine 3’,5’-cyclic monophosphate (cAMP). More than 1000 CFTR mutations have been described, with about half being due to missense mutations, 20% to splicing errors, and the remaining to nonsense and frameshift or promoter mutations. Clinical manifestations of CF are caused by impaired chloride conduction across epithelial cells, with diagnosis being confirmed by an elevated sweat chloride concentration (>60 mmol/L). The main cause of morbidity and mortality among CF patients is due to chronic airways obstruction and infection. Other manifestations include pancreatic obstructive insufficiency (85% of the patients), intestinal obstruction (5% to 10% of newborns), and liver disease (2% to 5% patients) (1–6).

About 98% of men with CF are infertile due to developmental abnormalities of the Wolffian duct that causes agenesis of the genital excretory ducts, including congenital bilateral absence of the vas deferens (CBAVD). CBAVD is responsible for about 2% of male factor infertility cases and 6% of obstructive azoospermia cases. Although CBAVD is considered a primary form of CF and the majority of the patients carry CFTR mutations, infertility due to CBAVD is not necessary coincident with CF.
clinical manifestations (7–12). In a review of 420 CBAVD cases, 19% carried two allele mutations, 47% a single mutation, and 34% had no identified mutation (13). The CFTR poly-T sequence located in the branch/acceptor splicing site of intron 8 (IVS8-T) has also been associated with CAVD. This IVS8-T exists in three variants, with 5, 7, or 9 thymidines. The 5T variant causes skipping of exon 9 with subsequent reduction in the functional CFTR protein. Due to the higher frequency of the 5T variant in CBAVD patients (21%) when compared with the general population (5%), the 5T variant is considered a CBAVD mutation with incomplete penetrance (14).

In the present study, we report the clinical features and mutational data of a CBAVD patient who was shown to be a compound heterozygote with a novel CFTR missense mutation.

MATERIALS AND METHODS
We extracted DNA from peripheral blood lymphocytes using a salting-out method. The initial CFTR screening included the 31 most common CFTR mutations within the Caucasian population, and was performed using a commercial kit (Cystic Fibrosis Diagnostic System, Applied Biosystems, Foster City, CA). The complementary screening of the whole CFTR gene (27 exons and flanking introns) was performed by denaturing gradient gel electrophoresis (CFTR-DGGE, Ingeny, The Netherlands). We amplified and sequenced the DNA samples with abnormal migration patterns in the DGGE analysis using the Big Dye Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems), and the products were analyzed in an ABI PRISM 310 Genetic Analyzer using the appropriate software (Sequencing Analysis, Applied Biosystems). Identification of poly-T variants at intron 8 (IVS8-T) was performed by polymerase chain reaction (PCR) using primers 19D9 (FAM-labeled) and E9R2. The PCR products were analyzed in an ABI PRISM 310 Genetic Analyzer with the appropriate software (GeneScan, Applied Biosystems). In all cases, informed consent was obtained for the genetic studies.

RESULTS
Clinical Data
The male white patient was born in 1958 (46 years old) at the Central region of Portugal. He had a past history of 19 years of infertility, first diagnosed in 1985. He married in 1983, and in 1985 his wife conceived a normal healthy child by intratruterine insemination with donor sperm. In 1995 he married a second time and was referred in 1996 to our in vitro fertilization (IVF) unit for infertility treatment. The spermogram showed azospermia with low semen volume and pH. The hematologic, biochemical, serologic, and hormonal status were normal.

The patient had a normal 46,XY karyotype, and no microdeletions were found in regions AZFa,b,c at Yq11.2. Bilateral absence of the vas deferens was suspected after physical examination and was confirmed by ultrasound and surgical exploration, with the bilateral diagnostic testicle biopsy sample showing conserved spermatogenesis. He had no scrotal pathologic conditions, other causes of testicular injury, or genital excretory duct obstruction; he did not take any medication and had no exposure to toxicants. The patient had a good general condition with no clinical manifestations or family history suggestive of CF, including pancreatic insufficiency, other gastrointestinal symptoms, or chronic airway disease. He presented with a normal sweat chloride concentration of 54 mmol/L.

In 1997, the couple underwent infertility treatment using testicle-retrieved sperm and intracytoplasmic sperm injection (ICSI). Seven metaphase II oocytes were retrieved and injected, of which six normally fertilized and cleaved, with two embryos having been transferred at day 2 (3B, 4B). A pregnancy was established followed by delivery of a normal healthy girl at 40 weeks of pregnancy. Preimplantation genetic diagnosis was not offered as no CFTR mutation was found in the patient’s wife.

Genetic Screening
The initial screening of the patient revealed a 7T/7T homozygote with absence of CFTR mutations. The whole gene was then screened by DGE, which showed altered product mobility for exons 17b and 20. Direct DNA sequencing of exon 17b identified a 3272-26A→G mutation. This mutation was detected in the mother of the patient after direct sequencing of exon 17b but not in the father, indicating that the mother is the carrier of the 3272-26A→G mutation. Analysis of exon 20 in the patient revealed a novel missense mutation, with a C to T nucleotide change at position 4000 (P1290S mutation) that leads to substitution of a proline by a serine in the CFTR protein (Fig. 1A, B). The nucleotide substitution creates a restriction site for the EcoRV enzyme that cuts the PCR amplicon of exon 20 (216 bp) into two fragments of 187 and 29 bp. Restriction analysis was thus used to screen and confirm the P1290S mutation as well as reveal that it was inherited from the father and not from the mother (see Fig. 1C).

To rule out the possibility of P1290S being a polymorphism, we analyzed 218 normal chromosomes from 109 fertile and healthy Portuguese males, 64 chromosomes from 32 patients with obstructive azoospermia due to congenital absence of the vas deferens (some of them with CFTR mutations identified), 30 chromosomes from 15 children carrying one CFTR mutation, and 10 chromosomes from five patients with confirmed CF (two CFTR mutations detected). In all cases, absence of the P1290S mutation was confirmed as no C→T was found at position 4000 of exon 20 in the CFTR gene. This mutation has not been previously reported in the CF Genetic Analysis Consortium (15).
DISCUSSION

Direct sequencing and DGGE in a CBAVD heterozygote patient for the 3272-26A→G mutation identified a novel P1290S CFTR mutation. The 3272-26A→G mutation accounts for about 2% of CF chromosomes in Portugal and has been described in compound heterozygotes for other CFTR mutations, which also explains the clinical variability observed among these patients (16). It originates an alternative acceptor-splicing site that results in a mRNA with 25 extra nucleotides from intron 17a and a premature stop codon. This alternative splicing site causes a reduction in the levels of normal transcripts and protein, but the remaining normal mRNA lessens the severity of the CF disease (17–20).

Several lines of evidence suggest that the present P1290S missense CFTR mutation might be pathogenic. First, it occurs in the nucleotide-binding domain 2 of the CFTR protein, which controls the opening of the channel (21–23). Second, as the 1290 codon corresponds to a highly conserved region that shows 100% amino acid sequence homology between species, the amino acid substitution is expected to substantially alter CFTR function (24, 25). Third, and although the isolated P1290S mutation had no substantial effect per se (the patient’s father is healthy and fertile), when associated with a mild CFTR mutation (P1290S/3272-26A→G) it caused a CBAVD phenotype in the present patient.

Patients who have a late diagnosis and a mild CF disease or congenital absence of the vas deferens often present one severe and one mild mutation such as 3272-26A→G (26). In particular, one compound heterozygote F508del/3272-26A→G patient had not developed lung disease or pancreatic insufficiency by 37 years of age, being genotyped only because of CBAVD (20). These data thus support the present findings, where the novel P1290S CFTR mutation was diagnosed at the age of 46 in an infertile CBAVD patient who had no clinical CF phenotype.

As CFTR mutations represent one of the most common abnormalities associated with CBAVD, a complete screening of the CFTR gene is of major importance for a proper genetic diagnosis. These findings are also relevant in regard to genetic counseling during infertility treatments. Partners of men with CFTR mutations should also be tested so that prenatal or preimplantation genetic diagnosis can be offered in cases where both are carriers.

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