

## ORIGINAL ARTICLE

# Significance of CFTR gene mutations in patients with congenital aplasia of vas deferens with special regard to renal aplasia

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Aplasia—CBAVD—cystic fibrosis—kidney—vas deferens

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## Summary

Between 1994 and 2010, a total of 123 patients with obstructive azoospermia due to aplasia of vas deferens (CAVD) were surgically treated. In 110 patients, the condition was bilateral (CBAVD), 13 men had unilateral aplasia (CUAVD), and 10 patients additionally had aplasia of one kidney. All patients underwent CFTR genetic testing, which detected two mutations (homozygous or compound heterozygous condition) in 38%, one mutation in 34% and no mutation in 28% of the patients with CBAVD. Neither the azoospermic patients with congenital unilateral aplasia of vas deferens nor those with CBAVD and renal aplasia were found to have CFTR mutations. The results militate against the assumption that there is an association between the CFTR gene and unilateral aplasia of vas deferens or bilateral aplasia of vas deferens with renal involvement.

## Introduction

Congenital aplasia of vas deferens (CAVD) is responsible for the clinical picture of obstructive azoospermia in 1–6% of cases (Young, 1949; Zielenski & Tsui, 1995; Attardo *et al.*, 2001; Samli *et al.*, 2006). Particularly in bilaterally affected patients, compound heterozygous or heterozygous mutations in the CFTR gene are considered a major cause, while controversy exists regarding patients with unilateral aplasia of vas deferens or those suffering from renal aplasia in addition to CAVD. Mutations in the CFTR gene are divided into five classes according to the severity (Zielenski & Tsui, 1995). When present in the homozygous condition, they cause cystic fibrosis, which is the most common genetic disease within the Caucasian population. The present study investigated whether CAVD is only associated with renal aplasia in the absence of CFTR mutations, as has often been postulated in the literature.

## Material and methods

The study comprised 123 patients, including 110 cases of CBAVD, five cases of unilateral (right) renal aplasia and 13 cases of CUAVD, of whom five patients had ipsilateral renal aplasia and obstructive azoospermia. Detailed patient and family history was taken with regard to renal

aplasia. In all patients, the clinical examination revealed unilateral or bilateral absence of vas deferens, which was later confirmed by scrotal exploration. All patients had normal testicular volume (>15 ml) and normal levels of follicle-stimulating hormone (FSH) (<8 mIU ml<sup>-1</sup>). Renal imaging was performed by ultrasound (3, 5 MHz). In nearly all cases, transrectal sonography (10 MHz) showed unilateral or bilateral absence of the seminal vesicles. With a view to the subsequent artificial reproduction, the patients and their partners underwent genetic testing to evaluate the possible hereditary risk due to CFTR mutations. Genetic testing was performed prior to sperm retrieval because the diagnosis was made in all patients during physical examination. Different institutes of genetics (Dr Ovens-Räder/Munich, Dr Klein, Dr Rost/Munich and others) evaluated the findings obtained by routine testing (33 mutations in the CFTR gene). After detailed genetic counselling, intracytoplasmic sperm injection (ICSI) was performed using spermatozoa that had been obtained by microsurgical epididymal sperm aspiration (MESA) in 78% of the patients, while testicular sperm extraction (TESE) had been carried out in all patients, also in those with MESA. Birth of a child was achieved in 76% of the couples (Schwarzer *et al.*, 2003).

The only two couples with a relevantly increased risk of cystic fibrosis because the women are heterozygous carriers decided against ICSI because of this risk.

**Results**

Mutations in the CFTR gene in patients with CAVD were distributed as follows:

**Patients with CBAVD**

Routine molecular genetic testing revealed homozygous or compound heterozygous CFTR mutations in 42 patients with CBAVD (38%), and 37 patients with CBAVD (34%) were shown to have a heterozygous mutation. DF508, the most common CFTR mutation worldwide, was detected in 61 patients, and the R117H missense mutation and the splice variant IVS8-5T were found in 22 patients each. The 3272-26A>G mutation was detected in two related patients with CBAVD, and L1388Q, W1282X and G551D in one patient each. All five patients with CBAVD and clinically manifest mucoviscidosis were shown to have a DF508 mutation. No gene mutation was detected in 31 patients (28%), including the five CBAVD patients with additional unilateral renal aplasia (Table 1).

Two mutations were found in 42 patients; the most frequently detected genotype was the compound heterozygous combination DF508/R117H in 17 patients with CBAVD (40%), followed by DF508/IVS8-5T in 13 patients (31%). In the heterozygous condition (*n* = 37 patients), DF508 mutation (21 patients, 57%) was followed by the IVS8-5T splice variant (six patients, 16%) and R117H mutation (six patients, 16%) (Table 2).

**Patients with CUAVD**

No CFTR mutations were detected in the patients with unilateral aplasia of vas deferens, independently of whether they had orthotopically located kidneys on both sides or ipsilateral renal aplasia, as was the case in five patients with CUAVD.

In summary, from our studies, it can be concluded that CFTR gene mutations are indeed significant in CBAVD patients without renal involvement; they were found to be a causative factor in 79 of the 105 patients (75%). However, our studies do not support a conclusion as to whether, and to what extent, these gene mutations are of importance in the patients with CUAVD and those with CAVD and concurrent renal aplasia. It can only be stated that genetic testing failed to find CFTR mutations in all these patients. The possible causes are discussed hereinafter.

**Discussion**

There are contradictory views on the probability of detection of CFTR mutations in the patients with CAVD. Costes *et al.* (1995) found at least one CFTR mutation in 93% of their patients, having excluded those with concurrent renal aplasia. On the other hand, despite mass spectrometric analysis of 100 CFTR mutations, Wang *et al.* (2002) identified only 36% CBAVD patients with two mutations and 31.5% with one mutation, while Attardo *et al.* (2001) and Hussein *et al.* (2011) found 40–48% of their patients with CBAVD to have a CFTR mutation.

The spectrum of the investigated CFTR mutations certainly plays an important role. During our 16-year study period, the initially less extensive mutation spectra were repeatedly expanded and updated, depending on the respective genetics institute. The later genetic testing was performed, the larger was the mutation spectrum, and the higher was the probability to detect possible CFTR mutations.

Another criterion for the evaluation of the probability of detection is the patient’s origin. There are worldwide variations in the prevalence of particular CFTR mutations. Therefore, according to the patient’s ethnicity, the mutation spectrum recommended by the Cystic Fibrosis Genetic Analysis Consortium should be used.

**Table 1** Frequency of CFTR mutations detected in 110 patients with CBAVD

Mutation:	DF508	R117H	IVS8-5T	3272-26A>G	L1388Q	W1282X	G551D
Patients with CBAVD	61 (55) <sup>a</sup>	22 (22)	22 (22)	2 (2)	1 (1)	1 (1)	1 (1)

<sup>a</sup>Percentages in brackets refer to total number of patients with CBAVD (110).

**Table 2** Frequency of genotypes detected in patients with CBAVD

DF508/R117H	DF508/IVS8-5T	Others	DF508	IVS8-5T	R117H	Others
17 patients with CBAVD (40) <sup>a</sup>	13 patients with CBAVD (31)	12 patients with CBAVD (29)	21 patients with CBAVD (57)	6 patients with CBAVD (16)	6 patients with CBAVD (16)	4 patients with CBAVD (11)

<sup>a</sup>Percentages in brackets refer to genotypes with two mutations (*n* = 42) or one mutation (*n* = 37) detected.

Unfortunately, this is not observed in most cases, so that the probability of detection often lies far below a tolerable level, especially in foreign patients – both CFTR mutations were only detected in two of our 15 CBAVD patients of non-German origin.

It is a striking fact that no CFTR mutations were detected in our patients with CUAVD and those with CAVD and concurrent renal aplasia. Most authors postulate that these two forms of CAVD are based on a particular ontogenetic aetiology independent of CFTR mutations (Ratbi *et al.*, 2007; Havasi *et al.*, 2010). However, this is contradictory to previous studies by Mickle *et al.* (1995), who demonstrated CFTR mutations in the patients with CUAVD, and those by Casals *et al.* (2000), who identified mutations in patients with CAVD and renal involvement after extensive genetic analysis. In our retrospective study performed between 1994 and 2010 with a comparably large number of patients, not a single one among those with unilateral or renal involvement was found to have a CFTR mutation. Considering the high detection rate for CFTR mutations in 75% of CBAVD patients without renal involvement, it appears unlikely that nondetection of mutations in the comparative group would be accidental. However, further detailed studies are required to provide evidence that bilateral rather than unilateral aplasia of vas deferens is associated with cystic fibrosis.

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### References

- Attardo T, Vicari E, Mollica F, Grazioso C, Burrello N, Garofalo MR, Lizzio MN, Garigali G, Cannizaro M, Ruvolos G, d'Ágata R, Calogero AE (2001) Genetic, andrological and clinical characteristics of patients with congenital bilateral absence of the vas deferens. *Int J Androl* 24:73–79.
- Casals T, Bassas L, Egozcue S, Ramos MD, Giménez J, Segura A, Garcia F, Carrera M, Larriba S, Sarquella J, Estivill X (2000) Heterogeneity for mutations in the CFTR gene and clinical correlations in patients with congenital absence of the vas deferens. *Hum Reprod* 15:1476–1483.
- Costes B, Girodon E, Ghanem N, Floris E, Jardin A, Soufir JC, Goossens (1995) Frequent occurrence of the CFTR intron 8 (TG)<sub>n</sub> 5T allele in men with congenital bilateral absence of the vas deferens. *Eur J Hum Genet* 3:285–293.
- Havasi V, Rowe SM, Kolettis PN, Dayangac D, Sahin A, Grangeia A, Carvalho F, Barros A, Sousa M, Bassas L, Casals T, Sorscher EJ (2010) Association of cystic fibrosis genetic modifiers with congenital bilateral absence of the vas deferens. *Fertil Steril* 94:2122–2127.
- Hussein TM, Zakaria NH, Zahran AM (2011) Clinical, laboratory and genetic assessment of patients with congenital bilateral absent vas deferens. *Andrologia* 43:16–22.
- Mickle J, Milunsky A, Amos JA, Oates RD (1995) Congenital unilateral absence of the vas deferens: a heterogeneous disorder with two distinct subpopulations based upon aetiology and mutational status of the cystic fibrosis gene. *Hum Reprod* 10:1728–1735.
- Ratbi I, Legendre M, Niel F, Martin J, Soufir JC, Izard V, Costes B, Costa C, Goossens M, Girodeon E (2007) Detection of cystic fibrosis transmembrane conductance regulator (CFTR) gene rearrangements enriches the mutation spectrum in congenital bilateral absence of the vas deferens and impacts on genetic counselling. *Hum Reprod* 22:1285–1291.
- Samli H, Samli MM, Yilmaz E, Imirzalioglu N (2006) Clinical, andrological and genetic characteristics of patients with congenital bilateral absence of vas deferens (CBAVD). *Arch Androl* 52:471–477.
- Schwarzer JU, Fiedler K, Hertwig I, Krüsmann G, Würfel W, Mühlen B, Pickl U, Löchner-Ernst D, Schleyer M, Ovens-Raeder A, Hennig M (2003) Male factors determining the outcome of intracytoplasmic sperm injection with epididymal and testicular spermatozoa. *Andrologia* 35: 220–226.
- Wang Z, Milunsky J, Yamin M, Maher T, Oates R, Milunsky A (2002) Analysis by mass spectrometry of 100 cystic fibrosis gene mutations in 92 patients with bilateral absence of the vas deferens. *Hum Reprod* 19:2066–2072.
- Young D (1949) Bilateral aplasia of the vas deferens. *Br J Surg* 36:417–424.
- Zielenski J, Tsui LC (1995) Cystic fibrosis: genotypic and phenotypic variations. *Annu Rev Genet* 29:777–807.