Letters to the Editor

Am. J. Hum. Genet. 61:1200–1202, 1997

CFTR Gene Mutations in Men with Bilateral Ejaculatory-Duct Obstruction and Anomalies of the Seminal Vesicles

To the Editor:

Mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene have a broad spectrum of phenotypic manifestations (Kerem and Kerem 1996; Stern 1997). Classical cystic fibrosis (CF) represents only the most severe end of the clinical spectrum. A host of milder CFTR-associated conditions exist, characterized either by a milder and less progressive natural course (Strong et al. 1991) or by limitation of manifestations to a single organ system. Congenital bilateral absence of the vas deferens (CBAVD) is an example of the latter category. In this condition, which presents clinically with obstructive azoospermia in otherwise healthy men, CFTR mutations are currently detected in 70%–80% of tested individuals (De Braekeleer and Férec 1996).

Men with CBAVD and also with full-blown CF commonly have various anomalies of the seminal vesicles, such as aplasia, hypoplasia, or cystic dilatation (Olson and Weaver 1969; Holsclaw et al. 1971; Honig et al. 1991). Also, autopsy studies have revealed that males with CF may have no anatomically discernible ejaculatory ducts (Olson and Weaver 1969; Holsclaw et al. 1971). Given the common embryological origin of these structures from the mesonephric ducts (Larsen 1993), it is not surprising to find combined abnormalities of vasa deferentia, epididymides, seminal vesicles, and ejaculatory ducts.

Some infertile men with anomalies of the seminal vesicles do not have concurrent vas deferens and epididymal malformations, a condition termed "isolated anomalies of the seminal vesicles" (IASV). Furthermore, seminal-vesicle anomalies may be encountered in association with bilateral ejaculatory duct obstruction (BEDO), another important cause of obstructive azoospermia (Pryor and Hendry 1991; Meacham et al. 1993). We hypothesized that, similar to CBAVD, IASV and BEDO with concomitant seminal-vesicle anomalies might represent two other CFTR-associated diseases confined to the male genital tract. We analyzed the CFTR gene in 23 patients with either of these two rare disorders.

All subjects presented for treatment of infertility. Nineteen of the tested individuals were German, two came from Turkey, one came from the former Yugoslavia, and one came from Italy. Evidence for seminal-vesicle dysfunction was obtained by semen analysis and transrectal ultrasound (Behre et al. 1997). Indicators of seminal vesicle dysfunction, obstruction, or aplasia are ejaculate that has subnormal volume (<2 ml), low fructose content (<13 μmol/ejaculate), and pH <7.2. Transrectal ultrasound was performed in most patients, to directly demonstrate anatomical anomalies of the seminal vesicles (Behre et al. 1995). For the diagnosis of IASV, three criteria needed to be fulfilled: (1) evidence of seminal-vesicle malformation, dysfunction or obstruction as described above, (2) exclusion of an obstruction of the seminal tract at the level of the vasa deferentia or epididymides, and (3) no evidence of major damage to the testicular parenchyma. Criteria (2) and (3) were demonstrated by clinical and ultrasound examination of the scrotal contents; measurement of α-glucosidase in seminal plasma, as a marker of epididymal function and patency (Cooper et al. 1990); by measurement of serum FSH; and, in selected cases, by surgical exploration of the scrotum and by testis biopsy. BEDO with concomitant seminal-vesicle anomalies was diagnosed when (*a*) the aforementioned criteria for IASV were fulfilled and (*b*) semen analysis showed azoospermia. If, under such circumstances, the scrotal parts of the vasa deferentia are palpable (or can be demonstrated to be intact on surgical exploration), this is pathognomonic of BEDO (Pryor and Hendry 1991).

DNA was isolated from peripheral lymphocytes, and target sequences were amplified by PCR. Direct testing by allele-specific amplification, heteroduplex analysis, or by restriction analysis was performed in all patients, for detection of the following CFTR gene mutations: R117H, R347P, ΔI507, ΔF508, 1717-1 G→A, G542X, G551D, R553X, 3849+10 kB, W1282X, and N1303K. The length of the polymorphic polythymidine tract (alleles T5, T7, and T9) in the intron 8/exon 9 splice-acceptor site was determined by sequencing. The T5 allele interferes with the splicing process of CFTR mRNA (Chu et al. 1993). It is common among men with CBAVD (De Braekeleer and Férec 1996) and may here,

in conjunction with a full-blown mutation, act as a mutation equivalent. If direct testing yielded no or only one mutation or T5 allele, the search was extended by denaturing gradient gel electrophoresis (DGGE [Costes et al. 1994]) comprising all 27 CFTR exons in the first seven patients and exons 3, 5, 6a, 8, 11, 12, 14a, 14b, 15, 17bi, 18, 20, 21, and 23 in the remaining 16. If abnormal bands were detected, direct sequencing of the respective exon followed.

Table 1 summarizes the results of the mutation analysis. Among the 16 patients from the IASV group, 1 subject was found to be heterozygous for the rare CF mutation I1139V (Teng et al. 1994), and another was found to be heterozygous for the T5 allele. In the BEDO group, six of seven men were mutation positive (when the T5 allele was considered as a mutation). Compound heterozygosity for ΔF508/R117H was detected in two patients, for ΔF508/T5 in another two, and for R553X/T5 in one. One man was heterozygous for R347P. For one of the five compound heterozygotes, parental DNA could be obtained. His father carries the ΔF508 mutation, and his mother carries the R117H mutation, proving that in the index patient the two mutations are in the trans phase.

Approximately 4% of healthy subjects from northern European populations carry a CF allele (Boat et al. 1989), and the prevalence of T5 is 3%–5% (De Braekeleer and Férec 1996). The two positive cases in the IASV group might well represent coincidental findings, and, given the absence of detectable mutations in the other 15 men from this group, it appears unlikely that IASV is caused by mutations in the CFTR gene. However, the possibility that IASV is an etiologically heterogeneous condition and that only a subgroup of these patients harbor CFTR mutations cannot be ruled out definitely. Six of seven men with BEDO and concomitant seminal vesicle anomalies were identified as carriers of CFTR-gene mutations. We suggest that these mutations are the molecular basis of the genital-tract anomalies and the resulting infertility in our patients. Similar to CBAVD, BEDO with concomitant seminal-vesicle anomalies thus may be regarded as a CFTR-associated disorder confined to the male genital tract. The spectrum of mutations encountered in CBAVD and in BEDO with concomitant seminal-vesicle anomalies overlaps considerably. R117H and the T5 allele are rarely found in full-blown CF, but they contribute to a major degree to the molecular pathology underlying both CBAVD (De Braekeleer and Férec 1996) and BEDO with concomitant seminal-vesicle anomalies.

In anatomical and etiological terms BEDO is a heterogeneous disorder (Pryor and Hendry 1991; Meacham et al. 1993); it may be either partial or complete, and only the latter leads to azoospermia. BEDO may be secondary to pelvic trauma and surgery, infections, prostatic neoplasms, or Müllerian-duct cysts (Pryor and Hendry 1991; Honig 1994). None of these possible etiologies could be identified among the cases from the present series. In our patients, both the obstruction of the ejaculatory ducts and the concomitant seminal-vesicle anomalies most likely represent primary malformations or functional abnormalities of mesonephric-duct origin.

Table 1

Summary of Mutation Analysis in CFTR Gene in 16 Men with IASV and in 7 Men with BEDO

Diagnosis	CFTR Genotype[a]	T5/T7/T9
IASV	+/+	7/7
IASV	+/+	7/7
IASV	+/+	7/7
IASV	+/+	7/7
IASV	+/+	7/7
IASV	+/+	7/7
IASV	+/+	7/9
IASV	+/+	7/7
IASV	+/+	7/7
IASV	+/+	7/7
IASV	+/+	7/7
IASV	+/+	5/7
IASV	+/+	7/7
IASV	+/+	7/7
IASV	I1139V/+	7/9
IASV	+/+	7/7
BEDO	ΔF508/+	9/5
BEDO	ΔF508/R117H	9/7
BEDO	+/+	7/9
BEDO	ΔF508/R117H	9/7
BEDO	R553X/+	7/5
BEDO	R347P/+	7/7
BEDO	ΔF508/+	9/5

[a] A plus sign (+) denotes the wild-type allele (i.e., no mutation was detected).

With the support of assisted-fertilization techniques, patients with BEDO are now able to father children (Silber et al. 1995). Their offspring must be regarded as being at increased risk for CF. The carrier frequency for CF is 4%–5% in many countries, so the female partner of an infertile man with BEDO may be heterozygous purely by chance. To recognize such a high-risk situation, especially when treatment with assisted reproductive technology is planned, CFTR-gene mutation analysis should be considered in patients with BEDO and concomitant seminal-vesicle anomalies.

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Acknowledgments

We thank the European Community Concerted Action for Cystic Fibrosis for providing amplification primers for the DGGE analysis. The technical assistance of Mrs. Britta Hitschfeld and Mr. Jürgen Wansch is gratefully acknowledged. This work was supported in part by Deutsche Forschungsgemeinschaft grant Ni-130/15-1.

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0002-9297/97/6105-0027$02.00

Am. J. Hum. Genet. 61:1202–1204, 1997

Mutations in the *TIGR* Gene in Familial Primary Open-Angle Glaucoma in Japan

To the Editor:

As described in an editorial by Raymond (1997), glaucoma is characterized by progressive excavation of the optic disk, with both loss of retinal nerve fiber and visual field defects. This disease is one of the most common causes of bilateral blindness, and it is estimated that by the year 2000 ~66.8 million people worldwide will be affected by it (Quigley 1996). Recently, the glaucoma gene*GLC1A* was shown to be identical to the trabecular meshwork–inducible glucocorticoid response (TIGR) gene (*TIGR*) (Stone et al. 1997). The *TIGR* gene was cloned by Polansky and colleagues (Nguyen et al. 1993; Polansky et al. 1997) and, also, was called "myocilin" (gene *MYOC*) when it was cloned by Kubota et al. (1997). Three different mutations in the gene were shown to be responsible for the development of primary open-angle glaucoma (POAG), the most common form of glaucoma (Stone et al. 1997). The prevalence of these mutations was reported to be 4.4% in familial POAG patients and 2.9% in unselected POAG patients (Stone et al. 1997). We investigated whether Japanese patients with familial POAG carry identical or other mutants on the same gene. As a result, two new mutations in the *TIGR* gene were found. The prevalence of mutations in the *TIGR* gene in Japanese patients with familial POAG was also investigated.

Peripheral blood samples were collected, with in-