CF Gene and Cystic Fibrosis Transmembrane Conductance Regulator Expression in Autosomal Dominant Polycystic Kidney Disease

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Abstract. Disease-modifying genes might participate in the significant intrafamilial variability of the renal phenotype in autosomal dominant polycystic kidney disease (ADPKD). Cystic fibrosis (CF) transmembrane conductance regulator (CFTR) is a chloride channel that promotes intracellular fluid secretion, and thus cyst progression, in ADPKD. The hypothesis that mutations of the CF gene, which encodes CFTR, might be associated with a milder renal phenotype in ADPKD was tested. A series of 117 unrelated ADPKD probands and 136 unaffected control subjects were screened for the 12 most common mutations and the frequency of the alleles of the intron 8 polymorphic Tn locus of CF. The prevalence of CF mutations was not significantly different in the ADPKD (1.7%, n = 2) and control (3.7%, n = 5) groups. The CF mutation was ΔF508 in all cases, except for one control subject (1717-1G→A). The frequencies of the ST, TT, and 9T intron 8 alleles were also similar in the ADPKD and control groups. Two additional patients with ADPKD and the ΔF508 mutation were detected in the families of the two probands with CF mutations. Kidney volumes and renal function levels were similar for these four patients with ADPKD and ΔF508 CFTR (heterozygous for three and homozygous for one) and for control patients with ADPKD collected in the University of Colorado Health Sciences Center database. The absence of a renal protective effect of the homozygous ΔF508 mutation might be related to the lack of a renal phenotype in CF and the variable, tissue-specific expression of ΔF508 CFTR. Immunohistochemical analysis of a kidney from the patient with ADPKD who was homozygous for the ΔF508 mutation substantiated that hypothesis, because CFTR expression was detected in 75% of cysts (compared with <50% in control ADPKD kidneys) and at least partly in the apical membrane area of cyst-lining cells. These data do not exclude a potential protective role of some CFTR mutations in ADPKD but suggest that it might be related to the nature of the mutation and renal expression of the mutated CFTR.

Autosomal dominant polycystic kidney disease (ADPKD) is the most common inherited nephropathy, with an estimated prevalence of 1/1000 among Caucasians. The disease is characterized by the development of multiple cysts within the kidney, which are inconsistently associated with extrarenal manifestations, including liver and pancreatic cysts and intracranial aneurysms. The typical course of ADPKD involves the slow enlargement of renal cysts over decades, leading to end-stage renal disease (ESRD) in the majority of patients (generally near the fifth decade of life) (1). One of the most striking features of ADPKD is substantial interfamilial and intrafamilial phenotypic heterogeneity, as evidenced by wide variability in the age at ESRD, ranging from infancy to old age (2,3).

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The first example of the effect of a modifying gene in human ADPKD is the association of the DD genotype of the angiotensin-converting enzyme insertion/deletion polymorphism with worse renal prognoses (14).

Several lines of evidence have demonstrated that abnormal fluid secretion across cystic epithelium is a key factor in ADPKD cyst progression (for review, see reference 15). This process seems to be driven by transepithelial, cAMP-regulated chloride secretion, which is mediated by cystic fibrosis (CF) transmembrane conductance regulator (CFTR), the protein encoded by the CF gene (recently renamed ABC7 for “ATP-binding cassette, subfamily C, member 7”) (16). CFTR is a 1480-amino acid integral membrane protein that contains an ATP-binding cassette domain and functions as both a chloride channel and a conductance regulator (17). CFTR mutations, resulting in functional defects in secretory epithelia (such as that in the respiratory tract, the intestine, or the exocrine pancreas), account for CF, which is the most common lethal autosomal recessive disease among Caucasians (16).

Although significant CFTR expression has been detected in the tubular epithelium lining various nephron segments in fetal and adult kidney (18–20), there is no overt renal phenotype in patients with CF (16). In contrast, the frequent occurrence of abnormalities of the Wolffian duct among subjects with various mutations or the T5 polymorphism in intron 8 of CF (21–23) suggests that CFTR is involved in the embryonic development of the male genital duct, a structure that includes the ureteric bud (24).

Strong CFTR expression in the apical area of ADPKD cyst-lining cells has been documented (25,26), and functional studies have demonstrated the role of CFTR in chloride secretion and cyst fluid accumulation (25,27). Therefore, a loss of CFTR function, such as occurs in CF, would be expected to slow cyst growth and attenuate the ADPKD phenotype. This hypothesis was supported by a recent report of a family with both ADPKD and CF (28); the ADPKD phenotype seemed to be less severe for the two patients affected by both ADPKD and CF, compared with members of the family without CF.

To investigate the potential disease-modifying role of the CF gene in ADPKD, we performed systematic screening for 12 of the most common mutations and for the intron 8 polymorphic Tn locus of CF in a large series of unrelated patients with ADPKD and control subjects. These analyses allowed the detection of ∆F508 mutations in two families with ADPKD, which were further investigated for genotype-phenotype correlations. A molecular mechanism that might account for these correlations is proposed, on the basis of the expression patterns of wild-type versus ∆F508 CFTR in ADPKD kidneys.

Materials and Methods

Patients with ADPKD and Control Subjects

The index patients from all families with ADPKD that were observed in the Division of Nephrology, St. Luc Academic Hospital (Brussels, Belgium), between September 1994 and December 1998 were included in this study. The diagnosis of ADPKD was established on the basis of familial history and detection of a minimal number of cysts in ultrasonographic examinations, according to age (29). Subsets of patients with ADPKD, i.e., slow and rapid progressors, were defined by the occurrence of ESRD before the age of 40 yr or after the age of 60 yr, respectively. Patients with known type 2 ADPKD were excluded from the study; therefore, our population consisted mainly of patients with type 1 ADPKD (1,3). Control subjects included individuals seeking genetic counseling (Center for Human Genetics, Brussels, Belgium), unrelated spouses of patients with ADPKD, and subjects used as control subjects in previous studies, without familial histories of ADPKD or CF. Informed oral consent was obtained from all patients and control subjects, and the use of DNA samples has been approved by the Université Catholique de Louvain Ethical Review Board.

DNA Extraction and Genotyping

DNA was isolated from peripheral blood lymphocytes using the NaCl extraction procedure and conventional techniques (30). Mutation and polymorphism analyses were performed with different PCR amplifications. Genomic DNA samples were screened using the Elucigene CF12 kit (based on Amplification Refractory Mutation System technology; Zeneca Diagnostics, Abingdon, UK), to detect the following 12 CFTR mutations: 1717-1G→A, G542X, W1282X, N1303K, ∆F508, 3849+10kbc→T, 621+1G→T, R553X, G551D, R117H, R1162X, and R334W. The characteristics of these mutations are shown in Table 1. The studied mutations accounted for approximately 85% of the alleles causing CF in the Belgian population (31,32).

The length of the intron 8 polythymidin tract (which may contain five, seven, or nine thymines, corresponding to the 5T, 7T, and 9T alleles, respectively) was investigated using the nested-PCR method, followed by electrophoresis (33). Exon 9 was amplified with primers CFTR.Exon 9.1 and CFTR.Exon 9.2 (34). The PCR conditions were as follows: denaturation at 95°C for 30 s, annealing at 52°C for 30 s, and extension at 72°C for 1 min, for 34 cycles. The reaction mixture contained 2.5 μl of PCR buffer (100 mM Tris-HCL, 15 mM MgCl₂, 500 mM KCl [pH 8.3]), 200 μM concentrations of each dNTP, 10 pmol of each primer, and 1 unit of AmpliTaq Gold DNA polymerase (Perkin-Elmer, Norwalk, CT), in a final volume of 25 μl containing 100 ng of genomic DNA. To amplify the polypyrimidine sequence, we performed a nested PCR with primers CFTR-1D9 and CFTR-E9R2 (22). The conditions for the nested PCR were as described above, except that 1 μl from the first PCR was amplified with AmpliTaq Gold DNA polymerase and annealing and extension were performed at 54°C for 30 s and 74°C for 40 s, respectively. The stretch was determined using the Gene Scan 672 software of a Perkin-Elmer/ Applied Biosystems 373A sequencer.

Linkage analyses for PKD1 and PKD2 were performed for the informative families of the slow progressor subset and for the two families with CF mutations (35,36). Linkage to the PKD1 locus was investigated with 5′-flanking markers, i.e., 16AC2.5 (D16S291) and CW2 (D16S663) in the telomeric position, VK5.b (D16S94) and 218EP6 (D16S246) in the centromeric position, and the KG8 CA repeat in the 3′ untranslated region of the gene (35). The microsatellites 16AC2.5, CW2, and KG8 were amplified using the conditions described previously (37); the former two were analyzed with GeneScan 672 software. Hybridization with the biallelic probes VK5.b (MspI) and 218EP6 (PstI) was performed by Southern blotting. Linkage to the PKD2 locus was analyzed with four multisatellite markers that flanked the gene on chromosome 4 (36).

Clinical Data and Assessment of Renal Volumes

The records for patients with ADPKD were reviewed for gender, geographical background (Belgium or southern Europe), age at the
Table 1. Characteristics of the 12 mutations of the CF gene screened for among the patients with ADPKD and the control subjects

<table>
<thead>
<tr>
<th>Name</th>
<th>Location</th>
<th>Nucleotide Change</th>
<th>CFTR Domain</th>
<th>Consequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>R117H</td>
<td>Exon 4</td>
<td>G→A at 482</td>
<td>TM2</td>
<td>Arg→His at 117</td>
</tr>
<tr>
<td>621+1G→T</td>
<td>Intron 4</td>
<td>G→T at 621+1</td>
<td>mRNA splicing mutation</td>
<td></td>
</tr>
<tr>
<td>R334W</td>
<td>Exon 7</td>
<td>C→T at 1132</td>
<td>TM6</td>
<td>Arg→Trp at 334</td>
</tr>
<tr>
<td>ΔF508</td>
<td>Exon 10</td>
<td>3-bp deletion between 1652 and 1655</td>
<td>NBD1</td>
<td>Phe-508 deletion</td>
</tr>
<tr>
<td>1717-1G→A</td>
<td>Intron 10</td>
<td>G→A at 1717-1</td>
<td>NBD1</td>
<td>mRNA splicing mutation</td>
</tr>
<tr>
<td>G542X</td>
<td>Exon 11</td>
<td>G→T at 1756</td>
<td>NBD1</td>
<td>Gly→Stop at 542</td>
</tr>
<tr>
<td>G551D</td>
<td>Exon 11</td>
<td>G→T at 1784</td>
<td>NBD1</td>
<td>Gly→Asp at 551</td>
</tr>
<tr>
<td>R553X</td>
<td>Exon 11</td>
<td>C→T at 1789</td>
<td>NBD1</td>
<td>Arg→Stop at 553</td>
</tr>
<tr>
<td>R1162X</td>
<td>Exon 19</td>
<td>C→T at 3616</td>
<td>NBD2</td>
<td>Arg→Stop at 1162</td>
</tr>
<tr>
<td>3849+10kbC→T</td>
<td>Intron 19</td>
<td>C→T in a 6.2-kb EcoRI fragment 10 kb from 19</td>
<td>NBD2</td>
<td>Creation of a splice acceptor site</td>
</tr>
<tr>
<td>W1282X</td>
<td>Exon 20</td>
<td>G→A at 3978</td>
<td>NBD2</td>
<td>Trp→Stop at 1282</td>
</tr>
<tr>
<td>N1303K</td>
<td>Exon 21</td>
<td>C→G at 4041</td>
<td>NBD2</td>
<td>Asn→Lys at 1303</td>
</tr>
</tbody>
</table>

a Modified from reference 16. TM, transmembrane domain; NBD, nucleotide-binding domain; CFTR, cystic fibrosis transmembrane conductance regulator.

Immunocytochemical Localization of CFTR in ADPKD Kidneys

Tissue blocks were prepared from three ADPKD kidneys, as described (19,25). The three samples were obtained at the time of nephrectomy for renal transplantation. One sample was obtained from the 33-yr-old son of index patient 2, who is affected with both type 1 ADPKD and CF (ΔF508/ΔF508) (Figure 1B, subject IV.3). Two control samples were obtained from patients with ADPKD (a 49-yr-old male patient and a 55-yr-old female patient) who tested negative for CF mutations. Before paraffin embedding, kidney samples were fixed for 12 h at 4°C in 4% paraformaldehyde (Boehringer Ingelheim, Heidelberg, Germany) in 0.1 M phosphate buffer (pH 7.4). Sections (6 μm thick) were rehydrated and incubated for 30 min with 0.3% hydrogen peroxide, to block endogenous peroxidase. After incubation for 20 min with 10% normal serum in phosphate-buffered saline, sections were incubated for 45 min with the anti-CFTR antibodies diluted in phosphate-buffered saline containing 2% bovine serum albumin. After washing, sections were incubated with the appropriate biotinylated secondary antibodies (Vector Laboratories, Burlingame, CA), washed again, and incubated for 45 min with avidin-biotin-peroxidase complex (Vectastain Elite; Vector Laboratories). All incubations were at room temperature in a humidified chamber. Control experiments included incubation in the absence of primary antibody or with control rabbit or mouse IgG (Vector Laboratories). Sections were mounted in glycerol/water and viewed with a Leica DMR photomicroscope.

Two antibodies, raised against different domains of human CFTR, were used. Polyclonal antibody 169 (a gift from W. B. Guggino, Johns Hopkins Medical School, Baltimore, MD) was raised against a peptide sequence within the R domain (residues 724 to 746), whereas monoclonal antibody MATG1031 (Transgène, Strasbourg, France) recognized a sequence in the first extracellular loop (residues 107 to 117). These two antibodies have been extensively characterized in human tissues, including normal and ADPKD kidneys (18,19,25).

Statistical Analyses

Data are presented as mean ± SD. Comparisons of results from different groups were performed using χ² and Fisher’s exact tests, as appropriate.

Results

Population Characteristics

Our series included 117 probands with ADPKD. The clinical characteristics of these patients are presented in Table 2, together with those of the control subjects. Within the ADPKD group, 20 and 15 patients met the criteria for slow and rapid progressors, respectively. Two-thirds of the patients with...
Figure 1. Pedigrees with PKD1 and CF genotypes. Patients with autosomal dominant polycystic kidney disease (ADPKD) are shown as filled symbols. The probands are indicated by the arrows. The different haplotypes of the PKD1 locus are shown along the vertical bars. The ΔF508 genotype and the intron 8 polymorphic Tn locus genotype are shown in the rectangles. −/−, wild-type; +/-, heterozygous ΔF508 mutation; +/+ homozygous ΔF508 mutation. (A) Pedigree of index patient 1. A patient with a history of uremia without clear demonstration of renal cysts is depicted as a hatched circle. For subject II.5, the PKD1 haplotype was inferred from the haplotypes of his wife and son. (B) Pedigree of index patient 2.
ADPKD had reached ESRD (mean age, 50 yr) by the time of DNA sampling. Gender ($\chi^2 = 3.08, P = 0.08$) and geographic background ($\chi^2 = 0.22, P = 0.63$) parameters were similar for the ADPKD and control groups.

**CF Genotyping**

All ADPKD probands and control subjects were screened for the 12 CF mutations detailed in Table 1. Two patients with ADPKD (one in the slow progressor subset) and five control subjects exhibited a heterozygous CF mutation, which was identified as $\Delta F508$ for the two patients with ADPKD and four control subjects and $1717\text{-}1\text{G} \rightarrow \text{A}$ for one control subject (Table 2). The prevalence of CF mutations in the control group (3.7%) is similar to that reported in the literature (31,32) and does not differ significantly from that observed for patients with ADPKD (1.7%) (Fisher’s exact test, 0.46).

All subjects were also genotyped for the intron 8 polymorphic Tn locus of CF (Table 3). The vast majority of ADPKD probands and control subjects harbored the 7T allele, with a distribution of the three alleles similar to that reported for the general population (21). Each patient with ADPKD or control subject who exhibited the $\Delta F508$ mutation also bore the 9T allele, at least in the heterozygous state. The one control subject with the $1717\text{-}1\text{G} \rightarrow \text{A}$ mutation was homozygous for the 7T allele; this association was previously reported (21).

Thirteen of the 117 ADPKD probands were heterozygous for $\Delta F508$ CFTR mutations, as well as further analyses of their families, are described below.

**Additional Studies of the Two ADPKD Families with $\Delta F508$ CFTR Mutations**

**Clinical Histories.** The index patient from pedigree 1 (Figure 1A, subject III.4) was a 46-yr-old man. The diagnosis of ADPKD was made fortuitously by ultrasonographic examination at 30 yr of age, during a sterility evaluation that

### Table 2. Clinical characteristics and frequency of the wild-type ($\Delta F508 -/-$) and mutated forms of the CF gene, in the heterozygous ($\Delta F508 +/-$) and homozygous ($\Delta F508 +/+ $) states, among ADPKD probands (including the slow and rapid progressor subsets) and control subjects

<table>
<thead>
<tr>
<th></th>
<th>ADPKD Probands</th>
<th>Control Subjects</th>
<th>Ref. 21</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\Delta F508 -/-$</td>
<td>136</td>
<td>117</td>
<td></td>
</tr>
<tr>
<td>$\Delta F508 +/-$</td>
<td>117</td>
<td>117</td>
<td></td>
</tr>
<tr>
<td>$\Delta F508 +/+ $</td>
<td>117</td>
<td>117</td>
<td></td>
</tr>
</tbody>
</table>

* A fifth control subject had a different mutated CF allele (1717-1G→A), yielding a 3.7% total prevalence of CF mutations among the control subjects. ESRD, end-stage renal disease; ADPKD, autosomal dominant polycystic kidney disease.

### Table 3. Allelic frequency of the 5T, 7T, and 9T alleles of the intron 8 polymorphic Tn locus of CF in the ADPKD and control groups, compared with that reported by Kiesewetter et al. (21) for 224 normal chromosomes

<table>
<thead>
<tr>
<th>Allele</th>
<th>Frequency (%)</th>
<th>ADPKD Probands</th>
<th>Control Subjects</th>
<th>Ref. 21</th>
</tr>
</thead>
<tbody>
<tr>
<td>5T</td>
<td>5.6</td>
<td>2.2</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>7T</td>
<td>77.3</td>
<td>85.7</td>
<td>84</td>
<td></td>
</tr>
<tr>
<td>9T</td>
<td>17.1</td>
<td>12.1</td>
<td>11</td>
<td></td>
</tr>
</tbody>
</table>
revealed poor-quality spermatozoa with mobility abnormalities (30% spermatozoa with progressive mobility). At the age of 35 yr, hypertension was detected, requiring treatment with atenolol and enalapril. At that time, serum creatinine levels were normal (1.2 mg/dl). At the age of 43 yr, serum creatinine levels were observed to be elevated (1.9 mg/dl) for the first time. The subsequent history included three episodes of cyst bleeding, without infection, and a coronary artery bypass graft. At the age of 45 yr, the serum creatinine levels for the patient were 4.3 mg/dl. His father (subject II.5) was also affected with ADPKD and began to undergo hemodialysis at the age of 54 yr. Two paternal uncles (subjects II.3 and II.13) and three paternal aunts (subjects II.2, II.10, and II.11) were also affected with ADPKD. His paternal grandmother (subject I.2) was reported to be dead as a result of uremia.

The index patient from pedigree 2 (Figure 1B, subject III.1) was a 63-yr-old man. The diagnosis of ADPKD was made at the age of 53 yr, during an evaluation for gross hematuria and lumbar pain. At that time, serum creatinine levels were normal. Two episodes of gross hematuria occurred subsequently. Serum creatinine levels were measured to be 2.9 mg/dl at the age of 59 yr and 4.8 mg/dl at the age of 60 yr; hemodialysis was initiated at the age of 61 yr. The patient experienced no known episodes of cyst infection or nephrolithiasis. His mother (subject II.2), one maternal aunt (subject II.3), and his maternal grandmother (subject II.2) died as a result of uremia, at the ages of 65, 50, and 70 yr, respectively. Index patient 2 has two sons with ADPKD (diagnosed for both by ultrasonographic and CT examinations), of age 37 yr (subject IV.1) and 34 yr (subject IV.3). Of note, subject IV.3 has both ADPKD and CF; the latter condition was diagnosed on the basis of a positive sweat test (performed because of repeated respiratory infections during childhood) and mutation analysis (see below). Because of the deterioration of his pulmonary condition, he underwent heart-lung transplantation at the age of 26 yr. At that time, serum creatinine levels were normal (0.6 mg/dl). After transplantation, renal function progressively deteriorated, leading to the initiation of hemodialysis at the age of 31 yr. Bilateral nephrectomy was performed at the time of renal transplantation, 1 yr later.

**Genetic Analyses.** Segregation analysis for PKD1 and PKD2 was performed for the two families with CF mutations. For both, linkage analysis allowed exclusion of PKD2 and was compatible with linkage to PKD1 (Figure 1).

Transmission of the ΔF508 mutation of the CF gene was examined in both families (Figure 2). The ΔF508 allele harbored by index patient 1 (subject III.4) was transmitted by his mother (subject II.6) (Figure 2A). The ΔF508 mutation harbored by index patient 2 (subject III.1) was observed in his two sons, in a heterozygous state in the elder (subject IV.1) and in a homozygous state in the younger (subject IV.3) (Figure 2B). Genotyping confirmed that their mother (subject III.2) harbored the ΔF508 mutation in the heterozygous state. All six patients from these families who were heterozygous (n = 5) or homozygous (n = 1) for the ΔF508 mutation harbored at least one 97 allele (Figure 1).

**Correlations Between CF Genotype and ADPKD Phenotype.** Renal function and volumes were assessed for the two patients with ADPKD and a heterozygous ΔF508 mutation (index patients 1 and 2). The decrease in renal function (expressed as 1/creatinine concentration with time) for these two patients was compared with that for five unrelated patients with ADPKD without the CF mutation, who were matched for age and gender (Figure 3A). The slope was steeper for both index patients than for ADPKD control subjects, i.e., −0.0042 versus −0.0028 for index patient 1 (Figure 3A, upper) and −0.0082 versus −0.0040 for index patient 2 (Figure 3A, lower). With the exception of the sterility documented for index patient 1, these two patients experienced classic courses of ADPKD. Representative CT scans obtained at the age of 45 yr for index patient 1 and 63 yr for index patient 2 are shown in Figure 3B. Note that hepatic cysts were absent for both patients.

When available, creatinine clearance and renal size values for patients with ADPKD and CF mutations were compared with data collected in the large database on patients with ADPKD at the University of Colorado Health Sciences Center (28) (Table 5). The index patient from pedigree 1 (subject III.4) was characterized by relatively poor renal function (only 16% of male patients with ADPKD of that age have lower renal function), together with very large kidneys (99th and 87th percentile for the left and right kidneys, respectively). The index patient from pedigree 2 (subject III.1) was in the average range for both parameters. His two sons exhibited similar impairments of renal function; the elder had smaller ADPKD kidneys than did his younger brother, who was affected by both ADPKD and CF. It must be noted that, for the latter, the renal function and volume parameters presented in Table 5 were recorded before the combined heart-lung transplant, i.e., before the initiation of cyclosporin-based immunosuppressive therapy. Six years later, at the time of nephrectomy (age of 32 yr), the kidneys were even more enlarged (left kidney, 1097 cm3, 93rd percentile; right kidney, 721 cm3, 62nd percentile).

**Pathologic Examination Results and Immunolocalization of CFTR.** The pathologic examination of the kidneys removed from subject IV.3 from pedigree 2 (who is affected by both ADPKD and CF) demonstrated polycystic kidneys with multiple dilations of Bowman’s spaces and glomerular cysts, as well as areas of atrophic tubules (Figure 4, A to D). Areas of noncystic parenchyma exhibited extensive fibrosis (sclerosis index, 4/6). Multiple small focal adenomas were identified within cysts; some cyst lumina were filled with neutrophils. No signs of acute cyclosporin toxicity were observed. Very mild arteriolar hyalinosis was present.

Immunohistochemical analysis demonstrated that a majority of cysts were stained for CFTR (Figure 4, E and F); that observation was confirmed by systematic counting within 40 randomly selected fields, which demonstrated a positive immunoreaction in 75% of the cysts (153 of 203 cysts) examined. Typical inter- and intracyctic heterogeneity in CFTR staining was observed, regardless of the anti-CFTR antibody used (Figure 4, G and H). No specific reactivity was observed when incubations were performed with control IgG (Figure 4I) or without primary antibody (data not shown). Strong CFTR
staining was also observed in some of the glomerular cysts (Figure 4J). The CFTR staining pattern was either diffusely intracellular or concentrated in the apical area (Figure 4, G, H, K, and L). These characteristics are similar to those observed for ADPKD kidneys from patients without detected \(CF\) mutations (Figure 4, M and N) and those previously reported (25). The percentage of cysts stained for CFTR was 52% (142 of 273 cysts) in the two ADPKD kidneys without \(CF\) mutations.

**Discussion**

The systematic screening for the 12 most common \(CF\) mutations in a large series of 117 unrelated ADPKD probands demonstrated that (1) the prevalence of \(CF\) mutations among patients with ADPKD is lower than that in the general population, although the difference does not reach statistical significance; (2) the existence of a \(\Delta F508\) mutation in a patient with ADPKD is not associated with a milder renal phenotype in either the heterozygous or homozygous state; and (3) the presence of the \(5T\) allele of the intron 8 polymorphic \(Tn\) locus, which is known to be associated with Wolffian duct abnormalities, is not associated with a milder ADPKD phenotype. In addition, the first detailed examination of kidneys from a patient with type 1 ADPKD and CF demonstrated not only a marked cystic phenotype but also the persistence of strong CFTR expression in the majority of cysts.

The relatively high prevalence of heterozygous \(CF\) mutations in the general population in European countries (16,32) is in keeping with the 3.7% prevalence of mutations observed for our control group. Similarly, the allelic frequencies for the \(5T\), \(7T\), and \(9T\) alleles of the intron 8 polymorphic \(Tn\) locus of \(CF\) observed for our control subjects, as well as the association between these alleles and the two \(CF\) mutations described here, confirm the findings of a previous report (21). The prevalence of \(CF\) mutations among our series of 117 ADPKD probands (1.7%) was more than two times lower than that among our control subjects (3.7%). Although this difference did not reach statistical significance, the question arises of whether the association of ADPKD with \(CF\) might have a lethal effect. The existence of sterility resulting from spermatozoa of poor quality for one of the subjects with a \(\Delta F508\) mutation (index patient 1) must be noted. Indeed, the frequency of heterozygous carriers of \(CF\) mutations is increased among men with congenital bilateral absence of the vas deferens (CBAVD), as well as among subjects with azoospermia resulting from other causes.
or with sperm of poor quality (39). The variable penetrance of the phenotype of infertility among heterozygous carriers of CF mutations could depend on the association with other variants of the gene, such as the intron 8 polymorphic Tn locus. Whereas allele 5T of this locus seems to be involved in CBAVD (21–23), in conjunction with CF mutations or other CF variants (40), the genetic background involved in the occurrence of fertility problems among male heterozygous carriers of CF mutations without CBAVD is still unknown.

The existence of a ΔF508 mutation in two ADPKD probands allowed us to identify two other patients with ADPKD who harbored the same mutation in the heterozygous state. With the exception of documented sterility for one patient, the ADPKD phenotypes of these four patients were unremarkable. Compared with the University of Colorado Health Sciences Center database, their kidney size and creatinine clearance values displayed wide variability, ranging from the 16th to the 99th percentile (Table 5). This suggests that a heterozygous ΔF508 mutation in these patients does not affect the ADPKD phenotype, which is in agreement with the report by O’Sullivan et al. (28).

Examination of our patient with ADPKD and a homozygous ΔF508 mutation demonstrated that kidney involvement was similar to that observed for patients with ADPKD without CF. This patient exhibited an average ADPKD phenotype, as evidenced by kidney volume (particularly in comparison with his affected brother), creatinine clearance values, and morphologic examination results (extended cystic involvement). This absence of a protective effect of CF on ADPKD is in contrast to a previous report (28). Several explanations might account for this discrepancy. First, in view of the significant number of CF variants and the potential modifying role of variants located...
Figure 4. Cystic phenotype and CF transmembrane conductance regulator (CFTR) expression in the kidneys from a patient with ADPKD and CF (ΔF508/ΔF508). (A) Representative CT scan, showing the severely enlarged multicystic kidneys of the patient with ADPKD and CF, at the age of 26 yr. (B to D) Low-power views of trichrome blue-stained sections obtained from one of those kidneys. At the time of nephrectomy (age of 32 yr), the kidney is massively invaded by cysts of different sizes (B and C), including several glomerular cysts (D). Magnification, ×3.2 in B; ×62 in C and D. (E and F) Sections stained with the polyclonal anti-CFTR antibody 169 (E) or the monoclonal antibody MATG1031 (F). A majority of cysts show positive immunoreactivity for CFTR. Note that section E is in series with section D. Magnification, ×62. (G to I) Serial sections stained with the anti-CFTR antibody 169 (G), the anti-CFTR antibody MATG1031 (H), or control rabbit IgG (I). Specific staining for CFTR is detected in some cysts, with intracystic heterogeneity, irrespective of the nature of the antibody. Note that at least part of the CFTR staining is concentrated in the apical area of the cyst-lining cells, whereas diffuse intracellular staining is observed in other cells. Magnification, ×350. (J and K) CFTR staining (antibody 169) in a glomerular cyst (J) and in cyst walls (K). Different staining patterns, including diffuse intracellular and apical immunoreactivity, are observed. Magnification, ×250. (L) CFTR staining (antibody MATG1031), showing distinct apical reactivity in cyst-lining cells. Magnification, ×360. (M and N) CFTR staining (antibody 169) in two control ADPKD kidneys without detected CF mutations. The staining patterns are heterogeneous, including both intracellular and apical area immunoreactivity, similar to those shown in G, H, K, and L. Magnification, ×250.
Table 5. Creatinine clearance values and kidney volumes for four patients with ADPKD and a ΔF508 CFTR mutation

<table>
<thead>
<tr>
<th>Pedigree</th>
<th>Subject No.</th>
<th>CF Genotype</th>
<th>Age (yr)</th>
<th>Creatinine Clearance (ml/min)</th>
<th>Kidney Volume (cm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Left</td>
</tr>
<tr>
<td>1</td>
<td>III.4</td>
<td>ΔF508 +/-</td>
<td>46</td>
<td>41 (P16)</td>
<td>2208 (P99)</td>
</tr>
<tr>
<td>2</td>
<td>III.1</td>
<td>ΔF508 +/-</td>
<td>64</td>
<td>31 (P41)</td>
<td>1487 (P78)</td>
</tr>
<tr>
<td>2</td>
<td>IV.1</td>
<td>ΔF508 +/-</td>
<td>38</td>
<td>107 (P64)</td>
<td>460 (P19)</td>
</tr>
<tr>
<td>2</td>
<td>IV.3</td>
<td>ΔF508 +/-</td>
<td>26</td>
<td>98 (P52)</td>
<td>505 (P52)</td>
</tr>
</tbody>
</table>

*Percentiles obtained from the database of the University of Colorado Health Sciences Center are indicated in parentheses. Percentiles for creatinine clearance values and kidney volumes indicate that X% of subjects have lower or equal creatinine clearance values or kidney volumes, respectively.

Creative clearance value obtained at the age of 59 yr.

inside (21,40) or outside (14,41) the CF gene, the discrepancy could be attributable to different genetic backgrounds in the subjects described by O’Sullivan et al. (28) and us. Second, it could be related to the specific nature of the CF mutations detected. Indeed, the two patients with CF described by O’Sullivan et al. (28) each harbored a mutation known to reduce CFTR mRNA levels (stop codon within exon 3 and severe reduction in transcription for E60X; abnormal splicing for 3849+10kbC→T) (16,28); for our ΔF508/ΔF508 patient, the problem involved abnormal protein folding, resulting in a presumed lack of CFTR at the apical membrane (42). Third, it is possible that the less severe phenotypes of the two subjects described by O’Sullivan et al. (28) were attributable to their younger age, compared with their heterozygous or wild-type relatives. This argument must be considered, keeping in mind that the aggregate median survival time for CF is still <30 yr (16).

In view of the well established role of CFTR in intracystic fluid secretion in ADPKD (15), the existence of a protective role of the ΔF508 mutation because of decreased expression of the mutated protein in cyst-lining epithelial cells was an attractive hypothesis. However, decreased expression of ΔF508 CFTR was demonstrated only in sweat glands and submucosal glands of the upper airways (43,44). The inference that decreased expression also occurs in other tissues was recently challenged by Kälin et al. (45), who demonstrated that the expression of ΔF508 CFTR is strikingly tissue-specific, ranging from undetectable in sweat glands to indistinguishable from that of the wild-type protein in the respiratory and intestinal tracts. These data suggest that the variable severity of CF in different organs might reflect heterogeneity of expression of the mutated CFTR. It is currently unknown whether the latter hypothesis applies to the kidney. However, given the lack of major renal involvement in CF (16,46), it is not unlikely that the expression of ΔF508 CFTR is normal or only moderately decreased in the kidney.

Our study substantiates this hypothesis, because immunocytochemical examination of CFTR in a kidney sample from a patient with type 1 ADPKD and CF demonstrated ΔF508 CFTR expression in approximately 75% of cysts. This fraction of CFTR-positive cysts is greater than that observed for patients with ADPKD without CF mutations (52%) and for patients with ADPKD in general (60%) (25). At the level of resolution achieved here, at least part of the ΔF508 CFTR immuno-reactivity is concentrated in the apical membrane area. The staining pattern, including the significant inter- and intracystic heterogeneity, is similar to that previously reported (25) and that observed in patients with ADPKD without CF mutations (Figure 4). Keeping in mind the limitations of a single-sample examination, it is tempting to argue that the lack of a protective effect of the ΔF508 mutation in ADPKD reflects the fact that mutated CFTR expression remains stable in ADPKD cysts. By analogy with the studies of Kälin et al. (45), our findings might suggest that the ΔF508 mutation does not significantly impair the processing of CFTR in the kidney.

The amount of CFTR mRNA without exon 9 depends on the number of thymines in the polymorphic Tn sequence of intron 8. The 5T allele causes an increase in the number of CFTR mRNA transcripts that lack exon 9, which leads to a nonfunctional protein (47). The 5T allele has been associated with various genital, respiratory, and pancreatic phenotypes (21,22,48). Our study is the first to investigate the distribution of these alleles in patients with ADPKD. As shown in Table 4, we did not observe evidence for a protective effect of the 5T allele in ADPKD. However, we did not find patients homozygous for this rare allele or patients with both a 5T allele and a classic CF mutation. We did not look for the presence of alleles belonging to other loci, such as (TG)m and M470V, the combination of which might explain the penetrance of some mutations (40). Definitive exclusion of a protective effect of a particular combination of CF alleles would be a daunting task, given the highly polymorphic nature of that gene (32).

Even if it is confirmed, the lack of effect of the ΔF508 mutation on ADPKD phenotypes does not exclude a potential modifying role of the CF gene in ADPKD. It merely indicates that subjects with the ΔF508 mutation are not good models for assessment of this effect, as suggested by the possibility of tissue-specific regulation of mutated CFTR expression (45) and our demonstration of high ΔF508 CFTR reactivity in ADPKD cysts. The effects of a CF mutation affecting the single-channel properties of CFTR, rather than its processing, would be very interesting to examine. However, because ΔF508 represents 66% of the mutated alleles throughout the world (32), the chance of identifying an association between...
ADPKD and a rare mutation of CF is extremely small. Considerations of the role of CFTR in cyst growth must also take into account alternative mechanisms of chloride secretion in ADPKD cysts. Most of the morphologic and functional studies that support the role of CFTR in ADPKD have documented a striking heterogeneity in CFTR expression in the cells or cysts examined (25,27). It is possible that the expression and activity of CFTR in some cyst-lining cells are sufficient only to maintain a filled cyst or, alternatively, that transepithelial chloride secretion in ADPKD is mediated by different types of transporters (15,25).

In conclusion, we performed a wide screening of ADPKD probands, to assess the effects of CF mutations on kidney involvement. We did not observe a protective effect of ΔF508 (the most common CF mutation) on the renal ADPKD phenotype. This was also true for the ST allele of the intron 8 polymorphic Tn locus, which has been previously associated with Wolfian duct abnormalities. Furthermore, we demonstrated, for the first time, nearly normal levels of expression of the ΔF508 CFTR in kidney cysts. These data do not exclude a potential protective role of some CFTR mutations in ADPKD but suggest that it might be related to the nature of the mutation and renal expression of the mutated CFTR.

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References