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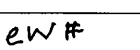
Insert word	Your proof	
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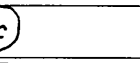
Make capital	your proof	
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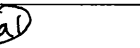
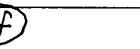
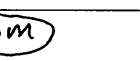
Make bold	Your proof	
Make roman (not italic or bold)	Your proof	
Let it stand; OK as is	Your proof	
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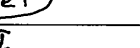
No paragraph	Marked. Your	
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Original Research

Genetic Analysis of Rwandan Patients With Cystic Fibrosis-Like Symptoms*

Identification of Novel Cystic Fibrosis Transmembrane Conductance Regulator and Epithelial Sodium Channel Gene Variants

Léon Mutesa, MD; Abul Kalam Azad, MD; Catherine Verhaeghe, PhD; Karin Segers, PhD; Jean-François Vanbellinghen, MSc; Louis Ngendahayo, MD; Emmanuel Kamanzi Rusingiza, MD; Philippe Rutwaza Mutwa, MD; Stephen Rulisa, MD; Lucien Koulischer, MD, PhD; Jean-Jacques Cassiman, MD, PhD; Harry Cuppens, PhD; and Vincent Bours, MD, PhD

Background: **The defect in chloride and sodium transport in cystic fibrosis (CF) patients is a consequence of CF transmembrane conductance regulator (CFTR) loss of function and an abnormal interaction between CFTR and the epithelial sodium channel (ENaC). A few patients were described with CF-like symptoms, a single CFTR mutation, and an ENaC mutation.**

Methods: **To study African patients with CF-like symptoms and to relate the disease to gene mutations of both CFTR and ENaC genes, we collected clinical data and DNA samples from 60 African patients with a CF phenotype. The CFTR gene was first analyzed in all patients by denaturing high-performance liquid chromatography followed by direct sequencing; whereas, the sodium channel non-voltage-gated 1 α (SCNN1A), sodium channel non-voltage-gated 1 β (SCNN1B), and sodium channel non-voltage-gated 1 γ (SCNN1G) subunits of the ENaC gene were analyzed by sequencing in the five patients who carried only one CF mutation. The frequency of all identified ENaC variants was established in a control group of 200 healthy individuals and in the 55 CF-like patients without any CFTR mutation.**

Results: **Three CFTR mutants, including one previously undescribed missense mutation (p.A204T), and a 5T/7T variant were identified in five patients. ENaC gene sequencing in these five patients detected the following eight ENaC variants: c.72T>C and p.V573I in SCNN1A; p.V348M, p.G442V, c.1473 + 28C>T, and p.T577T in SCNN1B; and p.S212S and c.1176 + 30G>C in SCNN1G. In the 55 CF-like patients without any CFTR mutation, we identified five of these eight ENaC variants, including the frequent p.G442V polymorphism, but we did not detect the presence of the p.V348M, p.T577T, and c.1176 + 30G>C ENaC variants. Moreover, these last three ENaC variants, p.V348M, p.T577T, and c.1176 + 30G>C, were not found in the control group.**

Conclusion: **Our data suggest that CF-like syndrome in Africa could be associated with CFTR and ENaC mutations.** ***(CHEST 2009; 135:1–??)***

Key words: Africa; cystic fibrosis; cystic fibrosis transmembrane conductance regulator mutations; epithelial sodium channel mutations

Abbreviations: CF = cystic fibrosis; CFTR = cystic fibrosis transmembrane conductance regulator; dHPLC = denaturing high-performance liquid chromatography; ENaC = epithelial sodium channel; PCR = polymerase chain reaction; PEM = protein energy malnutrition; PHA = pseudohypoaldosteronism; SCNN1A = sodium channel non-voltage-gated 1 α; SCNN1B = sodium channel non-voltage-gated 1 β; SCNN1G = sodium channel non-voltage-gated 1 γ; UTR = untranslated region; wt = wild type

Cystic fibrosis (CF) is an autosomal-recessive genetic disorder due to mutations in the CF transmembrane conductance regulator (CFTR) gene.[1] Very few CF cases have been described in African children[2,3]; this disease remains underdiagnosed in Africa because the CF phenotype is very similar to that of other frequent pathologies[5–8] and proper genetic analyses are inaccessible in many African countries.

On the other hand, mutations within the epithelial sodium channel (ENaC) genes are responsible for pseudohypoaldosteronism (PHA) type 1[9] and Liddle syndrome, a severe form of hypertension.[10] However, several studies have suggested that ENaC genes could be involved in CF-like disease, because transgenic mice expressing sodium channel non-voltage-gated 1 β (SCNN1B) in the bronchial epithelium have a CF-like airway disease[14] and some nonclassic CF patients have deleterious SCNN1B mutations.[15] We studied the CFTR gene in 60 African children with CF-like symptoms, and we explored the coding regions of the sodium channel non-voltage-gated 1 α (SCNN1A), SCNN1B, and sodium channel non-voltage-gated 1 γ (SCNN1G) genes encoding the α, β, and γ subunits of ENaC in patients with a single CFTR mutation.

*From the Department of Human Genetics (Drs. Mutesa, Verhaeghe, Segers, Koulischer, and Bours, and Mr. Vanbellinghen), Centre Hospitalier Universitaire-Sart-Tilman, GIGA-Research, University of Liège, Liège, Belgium; the Department of Human Genetics (Drs. Azad, Cassiman, and Cuppens), Katholieke Universiteit Leuven, Leuven, Belgium; the Department of Pathology (Dr. Ngendahayo), Centre Hospitalier Universitaire-Butare, National University of Rwanda, Butare, Rwanda; and the Department of Pediatrics (Drs. Rusingiza, Mutwa, and Rulisa), Centre Hospitalier Universitaire-Kigali, National University of Rwanda, Kigali, Rwanda.
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Correspondence to: Vincent Bours, MD, PhD, Department of Human Genetics, CHU Sart-Tilman, University of Liège, 4000 Liège, Belgium; e-mail: vbours@ulg.ac.be
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Materials and Methods

Patient Selection

We selected 60 unrelated Rwandan children with the following CF-like clinical features, on the basis of the 1998 Cystic Fibrosis Foundation Consensus Conference[16]: (1) suggestive clinical manifestations such as chronic sinopulmonary disease, GI manifestations (*eg*, pancreatic insufficiency, meconial ileus, diarrhea, vomiting, or constipation), failure to thrive, salt loss syndrome, and other manifestations such as diabetes mellitus or nasal polyps; (2) a history of CF in a sibling or a demonstration of abnormal nasal epithelial ion transport; and (3) a positive sweat chloride concentration. We also included isolated familial cases of severe protein energy malnutrition (PEM), which mimicked CF symptoms. In addition, we excluded HIV-positive patients and those with tuberculosis. We also excluded patients with PHA-1 and Liddle syndrome on the basis of normal BP and serum electrolyte levels in these patients. The study was approved by the Institutional Review Board, and peripheral blood samples were obtained from all subjects after informed consent was given.

CFTR Gene Analysis

All of the coding regions and intron/exon boundaries of the CFTR gene were amplified in a 50-μL reaction volume containing 0.5 mmol/L each primer, 500 μmol/L deoxyribonucleotide triphosphates (Roche Applied Science; Vilvorde, Belgium), a buffer (10X Fast Start buffer; Roche Applied Science), 1 to 2.5 mmol/L $MgCl_2$, and 2.5 units of a Taq DNA polymerase (Fast Start Ta DNA polymerase; Roche Applied Science). A mutation screening of the entire coding sequences of the CFTR gene was performed using denaturing high-performance liquid chromatography (dHPLC) analysis on a column (Transgenomic WAVE HPLC and DNASep Column; Transgenomic; Montluçon, France).[17–19] Abnormal dHPLC peaks were characterized by direct DNA sequencing (Big Dye Terminator Cycle Sequencing, version 3.1 kit; Applied Biosystems; Lennik, Belgium), using 16 ng of template, 3.2 pmol each primer, the sequencing buffer (Big Dye Terminator Cycle Sequencing, version 3.1 kit; Applied Biosystems), and the sequencing enzyme (RR-100 Sequencing enzyme; Applied Biosystems).

Site-Directed Mutagenesis and Expression of CFTR Mutant

The p.A204T mutation was introduced in the pcDNA3-CFTR expression plasmid (QuikChange XL Site-Directed Mutagenesis kit; Stratagene; La Jolla, CA). Polymerase chain reaction (PCR) reactions were performed under mutagenic conditions as previously described,[20] and the entire CFTR complementary DNA was sequenced to confirm the mutation.

Cell Transfection

HeLa cells (7.10^5 cells per 100-mm dish) were transfected with 5 μg of pcDNA3, wild-type (wt)-CFTR, F508del, or A204T plasmid (Transfectin; Bio-Rad Laboratories; Belgium).

Immunoblots

Seventy-five micrograms of total protein extracts were separated on 6% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and then transferred to a membrane (Immobilon-P Transfer Membrane; Millipore; Bedford, MA). These membranes were blocked for 1 h in a phosphate-buffered saline-

Tween solution containing 3% of nonfat dry milk, and were incubated overnight at 4°C in phosphate-buffered saline solution, 0.1% Tween 20, with 1 μg/mL mouse anti-CFTR antibody (clone MM13-4; Upstate; Huissen, the Netherlands). A secondary anti-mouse IgG antibody (Amersham Biosciences; Diegem, Belgium) was added, and protein bands were revealed using a reagent (ECL Western Blotting Detection Reagent; Amersham Biosciences).

ENaC Gene Analysis

All coding exons, and the exon/intron junctions, of the SCNN1A, SCNN1B, and SCNN1G genes, as well as the SCNN1A noncoding exon 1, were sequenced on both DNA strands, as described for the CFTR gene. ENaC gene pyrosequencing of eight variants (c.72T>C and p.V573I in SCNN1A; p.V348M, p.G442V, c.1473 + 28C>T, and p.T577T in SCNN1B; and p.S212S and c.1176 + 30G>C in SCNN1G) was performed in 200 healthy individuals and in the 55 patients without any CFTR mutation. PCR and pyrosequencing primers were designed using specific software (Pyrosequencing Assay Design Software, version 1.0.6; Biotage; Uppsala, Sweden). One of the PCR primers was biotinylated, which enabled easy immobilization of the PCR product on streptavidin-coated sepharose. Every PCR reaction was performed in a final volume of 50 μL containing 1 ng of genomic DNA, 1.5 mmol/L $MgCl_2$, 0.2 mmol/L deoxyribonucleotide triphosphates, 2 units of Taq polymerase (Roche Diagnostics; Mannheim, Germany), and 5 pmol each primer (Thermo Electro; Balen, Belgium). Twenty microliters of biotinylated PCR product was immobilized on streptavidin-coated sepharose high-performance beads (Amersham Pharmacia Biotech; Uppsala, Sweden) and was prepared with 70% ethanol, a denaturation solution, and washing buffers (Vacuum Prep Tool; Pyrosequencing; Uppsala, Sweden). The biotinylated templates were incubated with 0.4 μmol/L pyrosequencing primers at 80°C for 2 min in a PSQ96 plate. The sequencing was automatically performed on a pyrosequencer (Pyrosequencer PSQ 96; Biotage) at room temperature using reagents (PyroGold; Biotage).

Results

Clinical Presentation of African Children With CF-Like Symptoms

Sixty patients were recruited (27 boys, 33 girls). Their median age was 4.8 years (Table 1). The most common manifestations observed in these patients were PEM symptoms (87%), followed by the presence of GI symptoms (68%) and lung disease (65%). The majority of these patients (62%) had a positive sweat chloride test result (> 60 mmol/L), but, surprisingly, in seven patients the sweat chloride concentrations were > 120 mmol/L. Because it has been shown in previous studies,[6,21] this could be explained by the presence of PEM.

Genetic Variants of the CFTR Gene in Patients With CF-Like Disease

All of the coding regions of the CFTR gene were screened by dHPLC (Fig 1). We found 14 CFTR variants (Table 2), as follows: two known mutations (p.F693L and c.3120 + 1G>A); a novel p.A204T missense mutation; and nine sequence polymorphisms. In addition, two previously uncharacterized intronic nucleotide changes, c.3272–32T > C in the intron 17a and c.4575 + 2G>A in the 3′-untranslated region (UTR), were detected. The c.4575 + 2G>A change occurs after the last exon and is unlikely to alter CFTR function. Moreover, none of these intronic nucleotide changes altered a consensus splice site, suggesting that they are probably common polymorphisms rather than disease-causing mutations. The poly-T tract (Tn) near a poly-TG loci (TGm) in the branch/acceptor splicing site of intron 8 was analyzed in the five patients in whom one CFTR mutation had been identified (Table 3).

Patient P029 was heterozygous for the c.4575 + 2G>A intronic nucleotide change and had TG11T5 on one of his alleles. He presented mild GI symptoms with pancreatic insufficiency, failure to thrive, and severe symptoms of PEM. His sweat chloride concentration was positive (77 mmol/L).

The c.3120 + 1G>A mutation was identified in a 7-year-old girl (patient P007) presenting with respiratory infections due to *Pseudomonas aeruginosa* lung colonization, chronic diarrhea, and severe PEM symptoms. Her sweat chloride concentration was positive (85 mmol/L). She was heterozygous for the TG12T7 variant.

The p.F693L mutation was identified in the following two patients: a 13-year-old boy (patient P004) with recurrent respiratory infections, lung colonization by *P aeruginosa*, GI symptoms, failure to thrive,

Table 1—*Characteristics of 60 Patients With CF-Like Symptoms*

Variables	Patients (n = 60)	%
Age, yr		
Range	2–14	
Mean ± SD	5.8 ± 1.7	
Sex		
Male	33	55
Female	27	45
Phenotype		
Chronic lung disease	39	65
GI symptoms	41	68
Pancreatic insufficiency	19	32
Failure to thrive	23	38
PEM	52	87
Diabetes mellitus	4	7
Nasal polyps	3	5
Sweat chloride test results		
Positive (> 60 mmol/L)	37	62
Borderline (40–60 mol/L)	11	18
Normal (< 40 mmol/L)	9	15
Test not performed	3	5

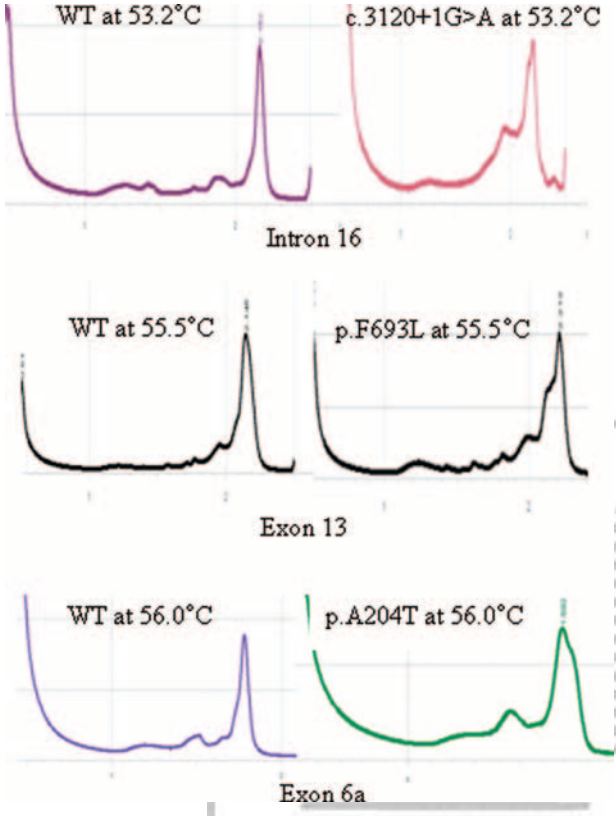

FIGURE 1. dHPLC chromatograms are shown with different conditions corresponding to various CFTR mutations from heterozygous patients compared to a normal control (wt). dHPLC profiles of CFTR mutants show extra peaks in contrast to the normal control.

diabetes mellitus, and PEM; and a 9-year-old girl (patient P038) with mild pulmonary symptoms, GI symptoms, and severe PEM. The sweat chloride concentrations were markedly increased in both patients (94 and 124 mmol/L, respectively). AQ: R

A 4-year-old girl (patient P041) with mild pulmonary disease associated with severe PEM status and a positive sweat chloride concentration (113 mmol/L) had a novel G>A mutation at nucleotide position 742 (Fig 1, 2 *top*, *A*), substituting a threonine for an alanine at amino acid position 204 (p.A204T). The comparison of CFTR protein sequences showed that amino acid 204 is highly conserved among vertebrates and also among human CFTR family members (Fig 2, *middle*, *B*). F2

To investigate its causative role in the CF phenotype, the expression of the p.A204T CFTR was studied after transient transfection of a p.A204T-CFTR-pcDNA3 expression vector in HeLa cells. The Western blotting (Fig 2, *bottom*, *C*) shows that only the core-glycosylated form of CFTR, referred to as *band B-130 kd*, was observed in cells expressing CFTR-F508del, while the wt-CFTR construct generated two forms, the band B (130 kd) and the band C (170 kd), which corresponds to the mature, glycosylated CFTR. Expression of the p.A204T CFTR allowed the expression of the mature glycosylated CFTR isoform at a level higher than that observed with the p.F508del mutant but reproducibly lower than the wt-CFTR level. In order to exclude the presence of a second CFTR mutation, the complete CFTR coding region and the exon-intron junctions were sequenced in these five patients in whom only one CFTR mutation had been identified, but we did not find any additional CFTR mutation. AQ: S

Genetic Variants of the ENaC Gene in Patients With One CFTR Mutation

The sequencing of SCNN1A, SCNN1B, and SCNN1G encoding the α, β, and γ subunits of the ENaC channel was performed in the five patients in whom a single CF mutant was identified. In total, eight ENaC sequence changes, including six novel variants, were identified (Table 3, Fig 3). Among these novel variants, there were two missense variants (p.V573I in the SCNN1A gene; p.V348M in the SCNN1B gene), two intronic nucleotide changes (c.1473 + 28C>T in the SCNN1B gene; c.1176 + 30G>C in the SCNN1G gene), one silent polymorphism (p.T577T in the SCNN1B gene), and a nucleotide change in the 5′-UTR (c.72T>C in the SCNN1A gene). In addition, two previously reported common polymorphisms were also identified (p.G442V in the SCNN1B gene; p.S212S in the SCNN1G gene). F3

The p.V573I ENaC variant was found in the patient who was heterozygous for the c.3120 + 1G>A CFTR mutation in combination with the TG12T7 variant, while the p.V348M ENaC mutation was observed in a patient with the p.F693L CFTR mutation. Interestingly, both patients had severe disease with *P aeruginosa* colonization and GI symptoms in combination with PEM signs (Table 3). The silent polymorphism p.T577T was found in a patient with a p.F693L CFTR mutation, whereas the c.72T>C was detected in the patient with the novel p.A204T CFTR mutation.

The two missense mutations, p.V573I (SCNN1A gene) and p.V348M (SCNN1B gene), lie in conserved areas (Fig 4). However, the p.V573I variation is a conservative sequence modification observed in other species and in control samples and is thus probably not functionally relevant, while the p.V348M amino acid change is not conservative and was not observed in any control (see below). This last mutation is thus probably relevant. F4 AQ: T

For the remaining 55 CF-like patients without any CFTR mutation, we searched for the presence of these eight ENaC variants identified in CFTR het-

Table 2—*Frequency of CFTR Sequence Variants in 60 Children With CF-like Disease (n = 120 alleles)*

Genotypes	Nucleotide Change	Exon/Intron	Consequence	Alleles, No.	CFTR Allele Variants, % (n = 120)
p.F693L	T>G at 2211	13	Phe>Leu at 693	2	1.67
c.3120 + 1G>A	G>A at 3120 + 1	Intron 16	Splicing mutation	1	0.83
p.A204T*	G>A at 742	6a	Ala>Thr at 742	1	0.83
c.4575 + 2G>A	G>A at 4575 + 2	3′ UTR		1	0.83
p.T854T	T>G at 2694	14a	Sequence variation	52	43.33
p.Q1463Q	G>A at 4521	24	Sequence variation	14	11.7
p.M470V	A>G at 1540	10	Sequence variation	13	10.83
c.1898 + 152T>A	T>A at 1898 + 152	12	Sequence variation	9	7.5
p.P1290P	A>G at 4002	20	Sequence variation	6	5
c.1001 + 11C>T	C>T at 1001 + 11	Intron 6b	Sequence variation	5	4.17
p.E527E	A>G at 1713	10	Sequence variation	3	2.5
c.2752 − 15C>G	C>G at 2752 − 15	Intron 14a	Sequence variation	3	2.5
c.3041 − 71A>G	G>C at 3041 − 71	Intron 15	Sequence variation	2	1.67
c.3272 − 32T>C	T>C at 3072 − 32	Intron17a		1	0.83

*Identified novel missense mutation.

T4 erozygous patients (Table 4). In this group, five variants, including the frequent p.G442V polymorphism, were identified, but we did not detect the presence of the p.V348M, p.T577T, and c.1176 + 30G>C ENaC variants. In addition, two patients carried the p.G442V polymorphism associated with the p.S212S and the p.V573I ENaC variants (data not shown).

For further investigations, we searched for the presence of all these ENaC variants in a control population of 200 ethnically matched volunteers
T5 (Table 5). Again, the p.G442V polymorphism was the most prevalent (11.3%) followed by the p.V573I variant (7.8%) and the p.S212S polymorphism (2%); whereas, the c.72T>C and c.1473 + 28C>T variants were detected in small proportions of these control subjects (0.5% and 0.8%, respectively). However, the missense mutation p.V348M was not found in the control group. We could also not find any p.T577T silent polymorphism or any c.1176 + 30G>C intronic nucleotide change in the control subjects.

Discussion

A diagnosis of CF is often difficult to assess in developing countries, because phenocopies are linked to malnutrition or frequent chronic infections such as HIV or tuberculosis, and many of these countries do not have any genetics laboratories. Therefore, despite several reports[3,4,22] of patients with CF, often associated with novel CFTR mutations, in Africa, the prevalence of CF in sub-Saharan Africa remains uncertain. However, African pediatricians regularly observe isolated cases of PEM in large families associated with recurrent lung infections, thus suggesting an autosomal-recessive transmission. AQ: U

Table 3—*Comparison of Clinical Findings in Five Patients With Identified CFTR and ENaC Mutants**

Patient/Sex/Age, yr	Phenotype	Sweat Test Value, mmol/L	CFTR Genotype	([TG]mTn) Genotype	ENaC Genotype (α, β, and γ Subunits)
P004/M/13	LD, PA, GI, PEM	94	p.F693L/−	(TG)10T7/(TG)11T9	p.V348M (β) p.S212S† (γ)
P038/F/9	LD, SA, GI, PEM	124	p.F693L/−	(TG)10T7/(TG)10T9	p.T577T (β) p.G442V† (β)
P007/F/7	LD, PA, GI, PEM	85	c.3120 + 1G>A/−	(TG)11T7/(TG)12T7	p.V573I (α) p.G442V† (β)
P029/M/2	GI, PI, FT, PEM	77	c.4575 + 2G>A‡/−	(TG)10T7/(TG)11T5	c.1473 + 28C>T (β) c.1176 + 30G>C (γ)
P041/F/4	LD, PEM	113	p.A204T/−	(TG)10T7/(TG)10T7	c.72 T>C (α 5′ UTR) p.G442V† (β)

*Tn = poly-T tract; TGm = poly-TG loci; LD = lung disease; PA = *P aeruginosa* lung colonization; FT = failure to thrive; DM = diabetes mellitus; PI = pancreatic insufficiency; SA = *Staphylococcus aureus* lung colonization; F = female; M = male.
†Known polymorphism.
‡Localized in 3′ UTR.

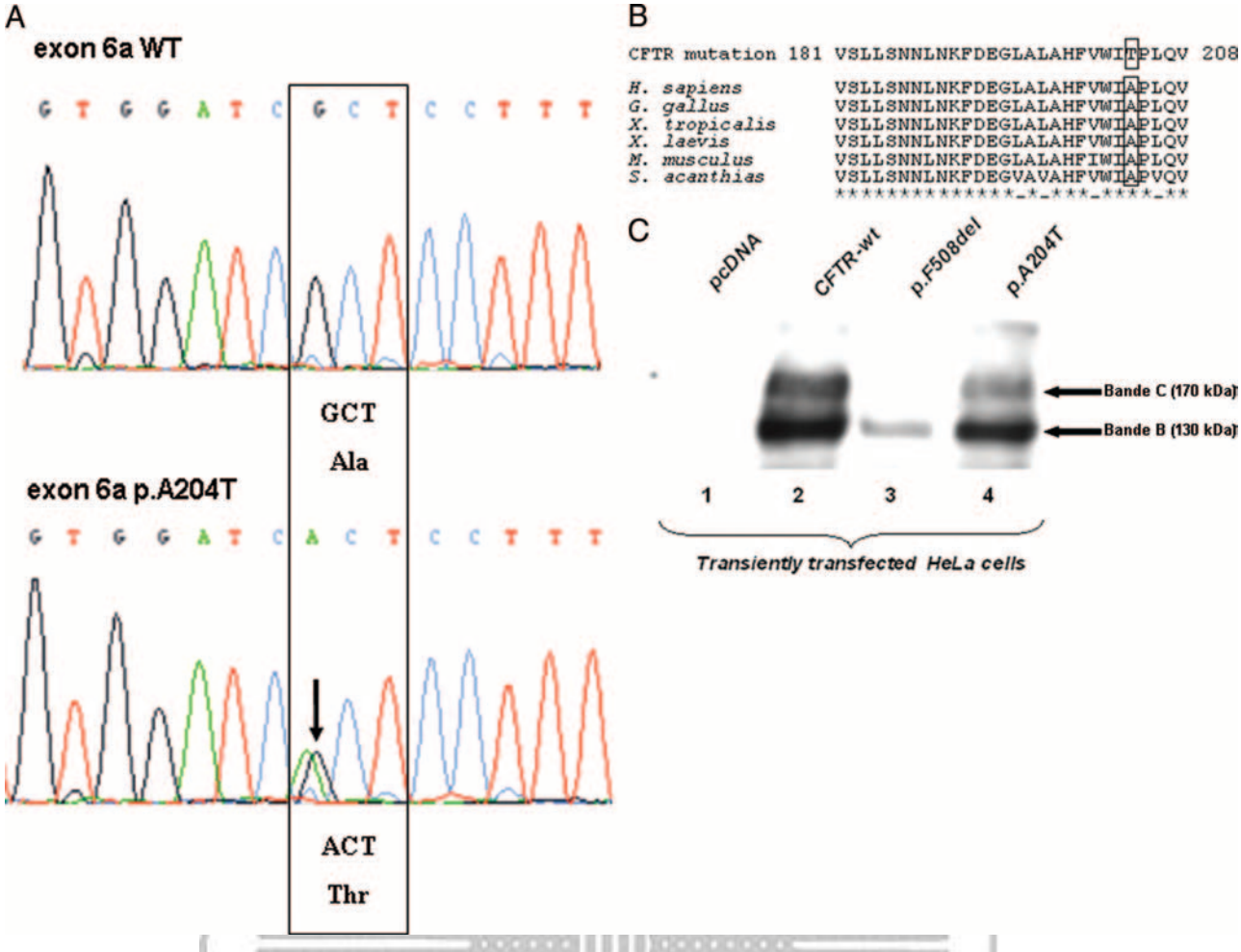

FIGURE 2. p.A204T missense mutation in the CFTR gene. *Top*, *A*: the electrophoregram shows the wt and A204T DNA sequence analyses of exon 6a. The nucleotide change (G>A) at 742 position and the codon (Ala to Thr) variation are shown. *Middle*, *B*: ClustalW multiple alignments of the CFTR regions containing aa 204, which is highly conserved among species. The T amino acid substitution is shown above the alignments. CFTR sequences were obtained from the Ensembl (http://www.ensembl.org). Sequence homology was compared using the ClustaW2 multiple sequence alignment. *Bottom*, *C*: Western blotting analysis of HeLa cells transiently expressing CFTR-wt, F508del, or A204T. HeLa cells were transfected with a pcDNA3 plasmid carrying each CFTR variant, as indicated (lanes 2 to 4), and with an empty pcDNA3 control vector (lane 1). On the right, the positions of bands B and C are indicated, as follows: band B, 130-kd core-glycosylated CFTR; band C, 170-kd mature glycosylated CFTR.

We selected 60 Rwandan patients on the basis of CF-like clinical symptoms. A sweat test was positive for CF in 37 patients (62%). However, a positive sweat test result cannot fully rule out a phenocopy as PEM can be associated with high Cl^- sweat values.[6,21] We therefore searched for CFTR mutations in all 60 patients through a dHPLC screening followed by a sequencing of positive exons. We detected only four patients with a single CFTR mutation and one patient with a TG11T5 variant. We did not observe any patient with a mutation on both alleles.

The identified mutations were as follows: (1) a c.3120 + 1G>A CFTR mutation, frequently observed in African patients and responsible for a splicing defect[4]; and (2) a p.F693L CFTR missense mutation in two patients with CF-like severe symptoms including recurrent lung disease (the same amino acid change at the same residue has been previously reported[23] in an Italian CF patient with severe symptoms; moreover, these two patients carried SCNN1B mutations; see below); (3) a TG11T5 variant, which can be associated with mild CF signs; and (4) a novel p.A204T CFTR mutation. This 204 residue is in the first membrane-spanning domain (MSD1), and almost all of the mutations within this region are associated with a mild phenotype, as was observed in our patient.[24,25] This missense mutation

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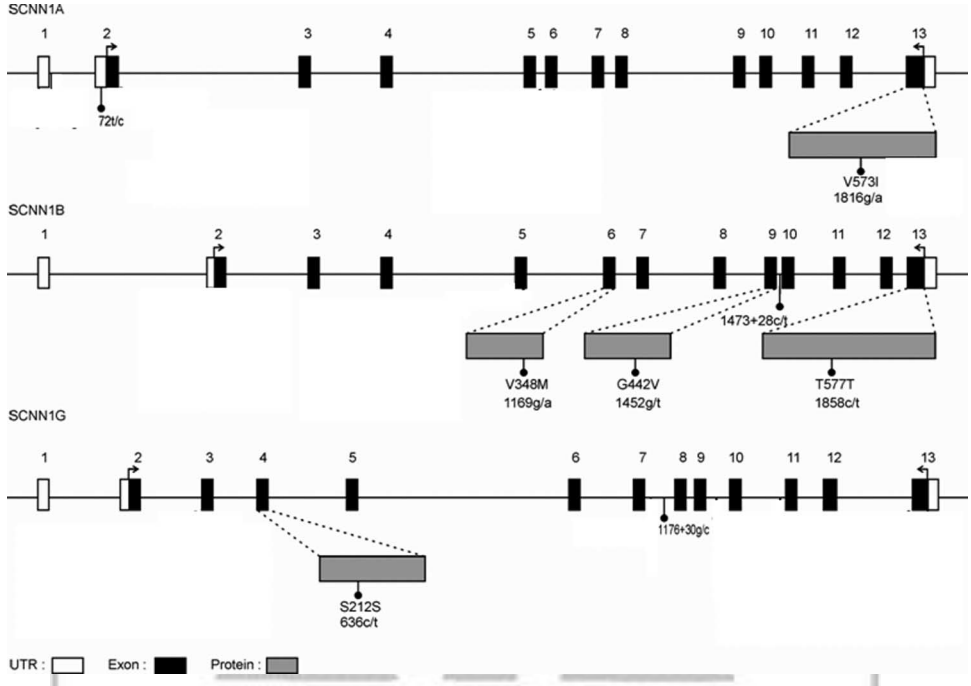

FIGURE 3. Schematic diagram of the different SCNN1A, SCNN1B, and SCNN1G mutations found in this study. The coding exons and their flanking 30 nucleotides in the introns were analyzed. The noncoding exon 1 was studied only for SCNN1A. Arrows = translation initiation and termination; open boxes = UTR; black boxes = coding exon; gray boxes = protein.

could be causative as it substitutes a conserved neutral hydrophobic amino acid (alanine) to a neutral polar amino acid (threonine). Exogenous expression of a p.A204T-CFTR protein in cells led to a weak expression and an altered protein glycosylation, but functional studies are required in order to assess precisely the consequences of this variant.

For the five patients carrying a single CFTR mutation or variant, we first sequenced the whole CFTR gene in order to exclude a second causal

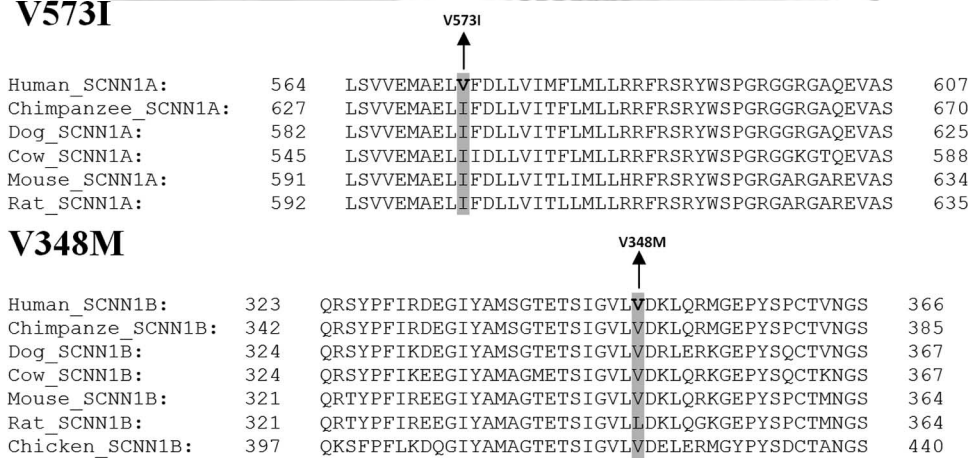

FIGURE 4. Orthologues and paralogues of the two ENaC subunits. The found missense mutations are highlighted in gray and marked above. SCNN1A and SCNN1B sequences were obtained from the National Center for Biotechnology Information database and were aligned with the ClustalW2 multiple sequence alignment program. Human (NP_001029.1, NP_000327.2), chimpanzee (XP_508948.2, XP_001161485.1), dog (XP_534912.2, XP_547092.2), cow (NP_777023.1, XP_612736.2), mouse (NP_03 5454.1, NP_035455.1), rat (NP_113736.1, NP_036780.1), and chicken (XP_425247.2) sequences are shown.

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Table 4—*Distribution of Eight ENaC Variants Among 55 CF-Like Patients*

	Variants							
Variables	c.72 T/C	c.1816G/A (p.V573I)	c.1169G/A (p.V348M)	c.1452G/T (p.G442V)	c.1473 + 28C/T	c.1858C/T (p.T577T)	c.636C/T (p.S212S)	c.1176 + 30G/C
Gene	αENaC	αENaC	βENaC	βENaC	βENaC	βENaC	γENaC	γENaC
Gene position	Exon 2	Exon 13	Exon 6	Exon 8	Intron 9	Exon 13	Exon 3	Intron 6
Frequent allele	T	G	G	G	C	C	C	G
Frequency on 110 chromosomes	0.991 (n = 109)	0.991 (n = 109)	1.00 (n = 110)	0.882 (n = 97)	0.991 (n = 109)	1.00 (n = 110)	0.982 (n = 108)	1.00 (n = 110)
Rare allele	**C**	**A**	**A**	**T**	**T**	**T**	**T**	**C**
Frequency on 110 chromosomes	0.009 (n = 1)	0.009 (n = 1)	Not observed (n = 0)	0.118 (n = 13)	0.009 (n = 1)	Not observed (n = 0)	0.018 (n = 2)	Not observed (n = 0)

Table 5—*Allele Frequencies of ENaC Variants as Determined in Control Group (n = 200)*

	Variants							
Variables	c.72 T/C*	c.1816G/A (p.V573I)	c.1169G/A (p.V348M)	c.1452G/T (p.G442V)	c.1473 + 28C/T	c.1858C/T (p.T577T)	c.636C/T (p.S212S)	c.1176 + 30G/C
Gene	αENaC	αENaC	βENaC	βENaC	βENaC	βENaC	γENaC	γENaC
Gene position	Exon 2	Exon 13	Exon 6	Exon 8	Intron 9	Exon 13	Exon 3	Intron 6
Frequent allele	T	G	G	G	C	C	C	G
Frequency on 400 chromosomes	0.995 (n = 398)	0.922 (n = 369)	1.00 (n = 400)	0.887 (n = 355)	0.992 (n = 397)	1.00 (n = 400)	0.98 (n = 392)	1.00 (n = 400)
Rare allele	C	A	A	T	T	T	T	C
Frequency on 400 chromosomes	0.005 (n = 2)	0.078 (n = 31)	Not observed (n = 0)	0.113 (n = 45)	0.008 (n = 3)	Not observed (n = 0)	0.02 (n = 8)	Not observed (n = 0)

*Localized in 5′-UTR.

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mutation, but we did not detect any of these. Several reports have indicated that the ENaC gene, coding for the amiloride-sensitive ENaC that is down-regulated by CFTR,[26] could also participate in the CF-like phenotype. Indeed, transgenic mice over-expressing SCNN1B develop a CF-like lung inflammation.[14] Moreover, several CF patients have been reported[27] who exhibited a CFTR mutation associated with an ENaC mutation. In our five patients, we identified several novel ENaC variants, including three that were not detected in a control cohort of 200 matched individuals. For instance, the two patients with a p.F693L CFTR mutation carried the p.V348M or p.T577T SCNN1B mutation, which were not detected in the control group. Moreover, the p.V348M mutation is not conservative and lies in a very conserved area (Fig 4). As reported in 2005,[15] CF patients with SCNN1B mutations can exhibit symptoms in the lungs and sweat glands without the renal features of PHA-1 or Liddle syndrome, thus suggesting that these mutations could increase ENaC activity and participate in the lung phenotype.

In the 55 patients who did not exhibit any CFTR mutation, we identified five ENaC variants that had been detected in the CF-like patients with a single CFTR mutation. Among these variants, the p.G442V polymorphism was the most frequent. Interestingly, a previous study[28] revealed that the p.G442V polymorphism was more prevalent in Africans (44%) than in white persons (1%), and functional studies[28,29] have indicated that this polymorphism is associated with increased ENaC activity. In addition, several other ENaC variant genes have been described[11,12,28,29] that have a higher prevalence in blacks than in whites, and most of them showed an association with hypertension. But, to our knowledge, none of these variants has been described in CF-like African patients without any CFTR mutation. Therefore, it is difficult to come to a definite conclusion on the relevance of these ENaC variants in our patients; however, our study opens the field for a large genetic and functional study of ENaC gene variants in African patients in order to explore whether, in association with an unfavorable environment, ENaC mutants could be sufficient to generate a CF-like phenotype with a positive sweat test result.

This is the first study that includes a clinical evaluation with CFTR and ENaC genetic analysis of CF-like Rwandan patients. It is difficult to conclude whether the association of a single CFTR mutation with an ENaC mutation or the presence of ENaC variants alone are sufficient to explain the symptoms. Possibly, in Central Africa, environmental conditions and mild CFTR and/or common ENaC mutations could have additive effects and generate symptoms that would not have been observed in a more favorable environment. In conclusion, the high incidence of ENaC mutations suggests that they probably play a role in our patients. Therefore, as other reports have demonstrated a high frequency of ENaC variants in Africans, it might thus be most important to perform the ENaC gene study in Africans with CF-like symptoms.

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beta-subunit of the epithelial Na+ channel in patients with a cystic fibrosis-like syndrome. Hum Mol Genet 2005; 14: 3493–3498
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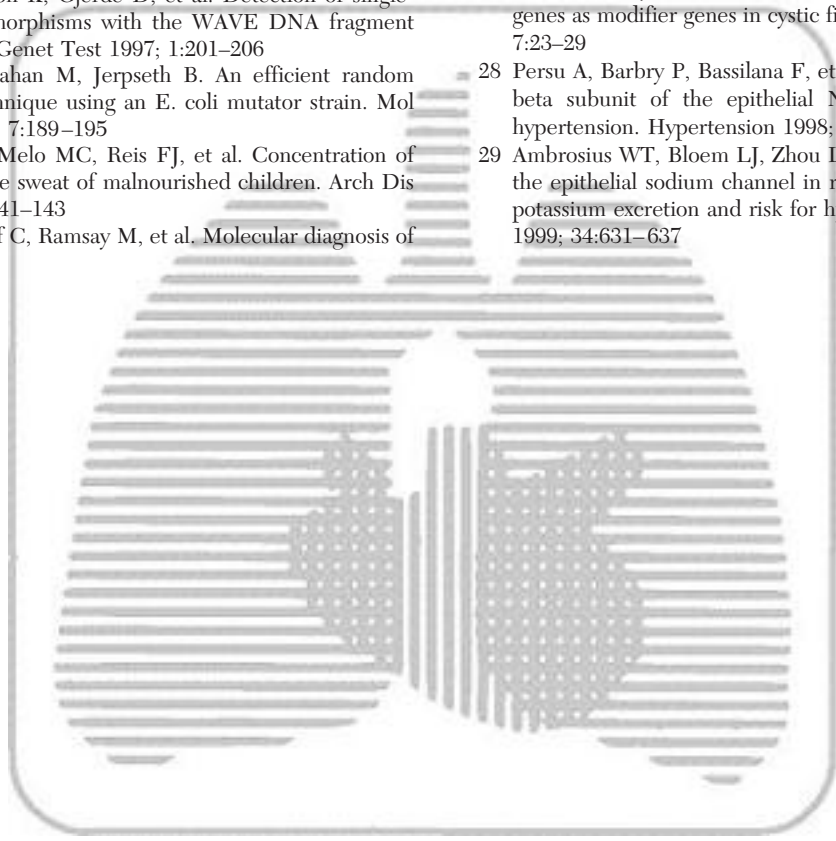

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