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Original Article

Assessment of epithelial sodium channel variants in nonwhite cystic fibrosis patients with non-diagnostic *CFTR* genotypes

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Abstract

Purpose: Several lines of evidence suggest a role for the epithelial sodium channel (ENaC) in cystic fibrosis (CF). The purpose of our study was to assess the contribution of genetic variants in the ENaC subunits (α, β, γ) in nonwhite CF patients in whom *CFTR* molecular testing has been non-diagnostic.
Methods: Samples were obtained from patients who were nonwhite and whose molecular *CFTR* testing did not identify two mutations. Sequencing of the *SCNN1A*, *B*, and *G* genes was performed and variants assessed for pathogenicity and association with CF using databases, protein and splice site mutation analysis software, and literature review.
Results: We identified four nonsynonymous amino acid variants in *SCNN1A*, three in *SCNN1B* and one in *SCNN1G*. There was no convincing evidence of pathogenicity. Whereas all have been reported in the dbSNP database, only p.Ala334Thr, p.Val573Ile, and p.Thr663Ala in *SCNN1A*, p.Gly442Val in *SCNN1B* and p.Gly183Ser in *SCNN1G* were previously reported in ENaC genetic studies of CF or CF-like patients. Synonymous substitutions were also observed but novel synonymous variants were not detected.
Conclusion: There is no conclusive association of ENaC genetic variants with CF in nonwhite CF patients.

Keywords: Cystic fibrosis; CF; Epithelial sodium channel; ENaC; *SCNN1*; Molecular diagnosis; *CFTR*; Nonwhite

1. Introduction

Cystic fibrosis (CF) is caused by mutations in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene, which encodes a chloride channel expressed predominantly in exocrine tissues [1]. The presence of two *CFTR* mutations typically results in chronic sinopulmonary disease and congenital absence of the vas deferens. There is considerable clinical heterogeneity and pancreatic, hepatic, and gastrointestinal manifestations also frequently occur [1].

The pathogenic mechanisms within lung tissue remain debated [2–11]. Two major hypotheses differ on altered salt concentration versus decreased serous secretions as the initial inciting incident affecting airway surface liquid, and leading to thickened mucus, decreased natural antibiotic function, and predisposition to infection [2–6]. A third hypothesis focuses on negative regulation of the epithelial sodium channel (ENaC) by CFTR in the lung [7–11]. In the context of CF, there is decreased chloride/bicarbonate (Cl^-/HCO_3^-) secretion due to loss of CFTR function and hyperabsorption of sodium (Na^+) and water (H_2O) due to increased ENaC activity with resultant dehydration of airway surface liquid and mucus thickening. These proposed pathologic mechanisms are neither exclusive nor necessarily exhaustive. In reality, more than one mechanism may contribute to the development of clinical manifestations.

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ENaC plays a role in salt and water resorption in epithelia in the lungs and in many additional organs, and is instrumental in the transition from newborn fluid to air-filled lung functioning [8–12]. It is heteromeric, consisting of alpha, beta and gamma subunits [13] encoded by the *SCNN1A*, *B* and *G* genes, respectively. There is also a variably expressed delta subunit present in some tissues, including lung epithelium [14,15]. Under-expression of ENaC due to mutations in the *SCNN1* genes leads to type 1 pseudohypoaldosteronism, a form of renal salt wasting, and overexpression to Liddle syndrome, a salt-sensitive hypertension [16]. A genetic association between ENaC and hypertension in African Americans has been reported [17].

The association of ENaC with lung pathology in humans is complex. In Liddle syndrome (ENaC overexpression), lung ENaC remains functionally inhibited by CFTR and pathology restricted to the kidney [18]. In pseudohypoaldosteronism (ENaC loss of function) there is increased fluid volume in lungs, and patients have poorly understood respiratory issues particularly at a young age (congestion, rhinorrhea, tachypnea, wheezing) [19]. These effects are compensated by increased mucociliary transport rates and respiratory issues tend to improve with age [19]. This supports ENaC involvement in lung processes but does not directly address ENaC overexpression in human lung disease. Mouse models with ENaC channel overexpression [20] or with ENaC protein levels increased by conditional knockout of ubiquitin ligase NEDD4L in lung epithelia [21] show a phenotype reminiscent of CF. To evaluate a potential role for ENaC in CF, several genetic studies in mostly white CF populations have been performed (Table 1). Stanke et al. examined 37 pairs of twins and their families and found transmission disequilibrium and intrapair discordance in support of roles for *SCNN1G* and *SCNN1B*, respectively [22]. Additionally, some patients with CF or CF-like disease but sequence changes in only one *CFTR* allele were found to have missense mutations in ENaC subunits [23–29]. To assess the functional effects of such missense changes, *Xenopus laevis* oocytes were injected with constructs containing specific mutations (p.Trp493Arg in α-ENaC (*SCNN1A*) and p.Val348Met, p.Glu539Lys, p.Pro267Leu, and p.Gly294Ser in β-ENaC (*SCNN1B*)) and exhibited altered sodium currents [23,28]. Further characterization indicated gain of function mechanisms in α-ENaC (*SCNN1A*) p.Trp493Arg through a reduction in the inhibitory effect of extracellular Na^+, and in β-ENaC (*SCNN1B*) p.Val348Met via the increased probability of ENaC channels remaining open [30,31].

Clinical genetic testing for CF can be performed by different approaches including mutation panel testing with panels that were primarily designed for carrier screening purposes, and DNA sequencing with or without deletion/duplication analysis. In composite, genetic testing does not identify a molecular cause in about 1–5% of typical CF patients, and a higher percentage in those with less classic presentations [32]. The elusive molecular etiology can be due to unidentified mutations in the *CFTR* gene itself, such as when sequence changes occur in noncoding regions that would not be included in diagnostic testing but that nevertheless harbor pathogenic changes, or it can result from mutations in other genes. For the "typical" CF patient, diagnosis can often be made based on newborn

Table 1
Summary of genetic association studies examining a potential role for ENaC in CF and CF-like conditions.

Ethnicity	# of subjects[a]	*SCNN1* subunit	Genotype/clinical characteristics[b]	Sequence variant[c]
Caucasian (France) [26]	56	B	Two *CFTR* mutations; Classic CF	p.Thr313Met, p.Gly589Ser
		G		p.Leu481Gln, p.Val546Ile
Caucasian (Europe) [28]	29; 47	A	One/no *CFTR* mutation; typical and atypical CF cases	c.−760A>G, c.−717G>C, c.−68C>T, p.Pro33Pro p.Phe61Leu, p.Val114Ile, p.Leu180Leu, p.Arg181Trp, **p.Ala334Thr**, p.Trp493Arg, **p.Thr663Ala**
		B		p.Ser82Cys, **p.Pro93Pro**, c.777-5T>C, **p.Phe293Phe**, p.Ile515Ile, p.Gly589Ser, p.Asp629Asp
		G		**p.Tyr129Tyr**, **p.Ile158Ile**, **p.Gly183Gly**, p.Glu197Lys, **c.1176+14A>G**, c.1373+29T>C, **c.1432−7G>A**, p.Leu649Leu
Unknown (USA) [23]	20	A	No *CFTR* mutations	p.Arg181Trp
		B	Non-classic CF	p.Glu539Lys, c.1543-2A>G
African 5; 55 (Rwanda) [27]		A	One/no *CFTR* mutation; CF-like	c.−28T>C, **p.Val573Ile**
		B		p.Val348Met, **p.Gly442Val**, p.Thr577Thr, c.1346+28C>T
		G		**p.Ser212Ser**, **c.1176+30G>C**
Caucasian (Spain) [29]	10	A	One/no *CFTR* mutation; CF and CF-like	p.Val14Gly, p.Leu203Leu, p.Arg204Trp, p.Ala304Pro, p.Ala357Thr, p.Cys641Phe, c.875+35G>A
		B		**p.Phe293Phe**, p.Arg563Gln, p.Pro574Pro
		G		**p.Gly183Gly**
Caucasian/African (France) [24]	20; 35	B	One/no *CFTR* mutation	p.Ser82Cys, p.Asn288Ser,p.Pro369Thr
		G	Bronchiectasis	**p.Gly183Ser**, p.Glu197Lys
Caucasian (Italy) [35]	24; 15	A	One/no *CFTR* mutation; CFTR-related disorders	c.−760A>G, **p.Ala334Thr**, **p.Thr663Ala**
		B		**p.Pro93Pro**, **p.Phe293Phe**
		G		**p.Tyr129Tyr**, **p.Ile158Ile**, **p.Gly183Gly**, **p.Leu649Leu**, **c.1176+14A>G**, c.1373+29T>C, **c.1432−7G>A**

[a] Listed as number of subjects per genotype (if known).
[b] Clinical characteristics listed are per original study description.
[c] Bolded variants were observed in the current study.