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Association of cystic fibrosis transmembrane conductance regulator gene variants with acute lung injury in African American children with pneumonia

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Abstract

Objectives—The cystic fibrosis transmembrane conductance regulator regulates fluid balance in alveolar epithelial cells and appears to modulate the inflammatory response. To determine whether more severe lung injury in children who develop community-acquired pneumonia is associated with variations known to affect function in the gene coding for cystic fibrosis transmembrane conductance regulator.

Design—A prospective cohort genetic association study of lung injury in children with community-acquired pneumonia.

Setting—Three major tertiary care children's hospitals.

Subjects—Caucasian and African American children with community-acquired pneumonia either evaluated in the emergency department or admitted to the hospital.

Interventions—None.

Measurements and Main Results—Caucasian and African American children with pneumonia were genotyped for the most common variants reported to affect cystic fibrosis transmembrane conductance regulator function, the p.508del mutation, the $(TG)_mT_n$ variable repeat region, and the M470V polymorphism in the cystic fibrosis transmembrane conductance regulator gene. Genotypes and haplotypes were determined, and the association of high-risk alleles or high-risk haplotypes (defined as the presence of at least one variant known to decrease the level of functional cystic fibrosis transmembrane conductance regulator) with the need for mechanical ventilation or the development of acute lung injury was evaluated. Forty-two children in the Caucasian cohort (n = 304) required mechanical ventilation; 32 developed acute lung injury. Forty-three children in the African American cohort (n = 474) required mechanical ventilation; 29 developed acute lung injury. In African American children, high-risk $(TG)_mT_n$ alleles known to

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result in decreased levels of functional cystic fibrosis transmembrane conductance regulator were associated with the need for mechanical ventilation (p = .0013) and the development of acute lung injury (p = .0061). Multivariable analysis demonstrated that high-risk $(TG)_mT_n$ alleles were independently associated with mechanical ventilation (odds ratios = 3.19; 95% confidence interval, 1.63–6.26) and acute lung injury (odds ratios = 3.36; 95% confidence interval, 1.50–7.53) in African American children.

Conclusion—Genetic variation in cystic fibrosis transmembrane conductance regulator is associated with acute lung injury in African American children with community-acquired pneumonia.

Keywords

acute lung injury; acute respiratory distress syndrome; genetic association study; pediatrics; pneumonia

Community-acquired pneumonia (CAP) is a common childhood infection. In Europe and North America, the annual prevalence of pneumonia in children aged <5 yrs is 34–40 cases per 1000 (1). Hospitalization rates of children with CAP remain high and complications from CAP may be increasing (2).

One complication of pneumonia is acute lung injury (ALI). Influx of fluid into the alveoli due to increased permeability of the alveolar-capillary barrier is one of the hallmarks of ALI (3), and the ability to clear fluid rapidly is associated with improved outcome (4). The clearance of alveolar fluid occurs through active ion transport (5), and the cystic fibrosis transmembrane conductance regulator (CFTR) has been shown to have a role in both cyclic adenosine monophosphate–stimulated fluid absorption and modulation of the epithelial sodium channel (6–8). CFTR is an ATP-binding cassette transporter chloride channel expressed on epithelial cells in bronchi, bronchioles, and alveoli (6, 7, 9, 10). The CFTR gene (*CFTR*) is a large gene with 27 exons that are spliced together to give mature CFTR messenger RNA. Alternatively spliced transcripts are relatively common and levels vary between individuals (11). Mutations in the *CFTR* gene cause cystic fibrosis (CF), a disease characterized by progressive injury to the lungs (12). *In vitro* and *ex vivo* studies indicate that CFTR deficiency results in a dysregulated inflammatory response (13–18) and promotes lipopolysaccharide-induced lung injury in mice (16, 19).

Genetic variants impact CFTR function. The most common mutation in *CFTR* is the deletion of phenylalanine 508 (p.508del) in exon 10, part of the first nucleotide-binding domain; inheriting two p.508del mutations causes CF (12). This mutation disrupts the processing and functioning of CFTR by causing mis-folding and retention in the endoplasmic reticulum (20, 21). Individuals with CF have <5% of normal CFTR activity (12). Only two common polymorphisms have been reported to affect the function of CFTR. One such polymorphism is the $(TG)_mT_n$ variable repeat region located in intron 8. Both *in vitro* and *in vivo* studies demonstrate association of either a higher number of TG repeats and/or a lower number of Ts with an increased proportion of messenger RNA transcripts missing exon 9 (22–26). Mechanistic studies also indicate that different alleles at the $(TG)_mT_n$ site likely affect exon 9 skipping due to differences between alleles in affinity of

the binding of splicing regulatory proteins (27, 28). Exon 9 is essential for CFTR function as it, together with exons 10–12, encodes for the first nucleotide-binding domain, and messenger RNA transcripts without exon 9 do not produce functional CFTR (20, 29, 30). In healthy individuals, 5–90% of the CFTR transcripts are missing exon 9 (31), suggesting that CFTR activity present in healthy individuals varies over a broad range. Although the reduction of CFTR activity to <5% of normal is observed in patients with CF, it is possible that other variants that reduce the level of functional CFTR to a lesser degree may still impact the risk of other lung disease (12).

Another polymorphism reported to be associated with variable levels of functional CFTR is the M470V polymorphism in exon 10. The methionine variant has been reported to have a 1.7-fold increase in intrinsic chloride channel activity *in vitro* (24). However, this has only been reported in this one *in vitro* study and the finding is complicated by reports that the M470V site is in significant linkage disequilibrium with the $(TG)_mT_n$ site (32, 33).

We hypothesized that children with CAP who do not have CF but have a genetic variation known to decrease functional CFTR will be more likely to have lung injury due to impaired clearance of alveolar fluid and/or a dysregulated inflammatory response. The association of haplotypes generated from the above-described sites (p.508del mutation, $(TG)_mT_n$ variable repeat region, and M470V polymorphism), or genotypes at the individuals sites, with either the need for mechanical ventilation or the presence of ALI was examined in Caucasian or African American children with CAP.

METHODS

Study Design

We performed a prospective, case-control genetic association study of genetic variation in the *CFTR* gene in pediatric patients with CAP to examine whether there was an association with the severity of disease. Outcome measures included the need for mechanical ventilation (MV, used as a surrogate marker of lung injury) or the development of ALI defined by the American European Consensus Committee definition (Pao_2/Fio_2 <300 with acute onset of bilateral infiltrates on chest radiographs and without the evidence of left atrial hypertension) (34). Children presenting to the emergency room or admitted to the hospital at three pediatric tertiary care centers were eligible for enrollment (Le Bonheur Children's Hospital, Children's Memorial Hospital, or Children's Hospital of Wisconsin). The Institutional Review Board from each institution approved the study. Some members of this cohort have been used in studies reported in previous publications (35–37). As the frequency of genetic polymorphisms and the linkage disequilibrium (LD) patterns differ between ethnicities and races (38, 39), such groups should be analyzed separately (40). Only the two largest subgroups, non-Hispanic Caucasians and African Americans (35% and 52% of the cohort, respectively), had a sufficient number of individuals for meaningful analysis. Race was self-reported.

A priori power calculations using Quanto 1.2.3 (41, 42) and published population estimates indicated that the cohort was powered (using 80% power) to observe associations giving minimal detectable odds ratios for high-risk haplotypes or $(TG)_mT_n$ alleles of 2.2 for

Caucasian and African American children for the need for MV, and of 2.35 for Caucasian and of 2.5 for African American children for ALI: the M470V site of 1.9 and 2.1 for MV and ALI in Caucasians and 2.3 and 2.6 for MV and ALI in African American children. The minimal detectable odds ratios were substantially higher for the p.508del site in the Caucasian population (minimal detectable odds ratios of 3.7 and 4.2 for MV and ALI, respectively). The p.508del site was primarily genotyped to include it in the haplotype analysis.

Study Population

Inclusion and exclusion criteria as well as data collection for children with CAP have been described in detail in previous publications (35–37). In brief, CAP was defined by 1) an acute illness (<14 days of symptoms); 2) the presence of a new chest radiographic infiltrate or consolidation confirmed by a radiologist; and 3) clinical features compatible with pneumonia, including one of the following: fever >37.8°C, hypothermia <36°C, peripheral white blood count >10,000/μL or <4500/μL, or >15% immature neutrophils; and two of the following: tachypnea (respiratory rate >2 standard deviations from the mean for age), dyspnea, or hypoxemia (pulse oximetry ≤94% on room air on initial evaluation without a known mixing heart lesion). Children who were not either African Americans or non-Hispanic Caucasians were excluded from analysis (as described above). In addition, children with congenital heart disease, severe bronchopulmonary dysplasia (defined as children on home oxygen therapy), or CF were excluded. Whole blood from healthy African Americans obtained from the blood bank was used to genotype the TG_mT_n site.

Genotyping

DNA was extracted from whole blood using the Wizard Genomic DNA Purification Kit (Promega, Madison, WI). Genotyping was performed by those blinded to the clinical status of individuals. Genotyping of the p.508del (rs332) and M470V (rs213950) alleles was performed using a 5′ nuclease TaqMan assay (Applied Biosystems, Foster City, CA).

To determine the genotype of the $(TG)_mT_n$ site, the region was amplified using a nested polymerase chain reaction strategy similar in design to that described previously for genotyping of the T_m region (43); however, primers were designed to allow sequencing across the $(TG)_mT_n$ site. The second polymerase chain reaction product was treated with Exo SAP-IT(Affymetrix, Santa Clara, CA) and sequenced (Retrogen, San Diego, CA) in the forward direction with the primer 5′CATCATGTCCTCTAGAAACCG3′; samples that were heterozygous for $(TG)_mT_n$ alleles of different length were also sequenced in the reverse direction with the primer 5′GGATCCAGCAACCGCCAACA3′. Two individuals independently read the sequences; there was a complete agreement on $(TG)_mT_n$ allele calls from the sequences.

For each of the three sites, ~10% of randomly chosen samples were genotyped a second time to verify reproducibility; there was 100% agreement in calls. Genotype frequencies of the p.508del and M470V sites did not deviate from Hardy-Weinberg equilibrium in any group. Genotype frequencies at the $(TG)_mT_n$ site did not deviate from Hardy-Weinberg equilibrium in the healthy African American group but did deviate from equilibrium in Caucasian (p = .

0012) and African American (p = .0079) children with CAP (determined using Genepop 4.1.1, http://genepop.curtin.edu.au/).

Haplotype Determination

Haplotypes were determined using PHASE 2.1 (http://www.stat.washington.edu/stephens/phase/download.html) (44, 45).

Linkage Disequilibrium

LD between the $(TG)_mT_n$ site and either the M470V or p508del sites was determined using the method of Zaykin et al (46) for sites with greater than two alleles.

Statistical Analyses

Caucasian and African American children were analyzed separately. A two-sided Fisher's exact test was used to calculate the statistical significance of categorical variables and univariate allele and haplotype analyses. An exact Cochran-Armitage test for trends was used to evaluate whether MV or ALI was associated with an increasing number of high-risk $(TG)_mT_n$ alleles. A nonparametric Mann-Whitney test was performed for the continuous variable, age. To compare the $(TG)_mT_n$ allele frequencies between healthy African Americans and African children with CAP, a Fisher-Freeman-Halton test was done using Cytel StatXact (Cytel Inc., Cambridge, MA). Multivariable logistical analysis was performed using SAS 9.2 (SAS Institute, Cary, NC). This multivariable analysis used logistic regression models created with either the need for MV or the development of ALI as the dependent variable. (When ALI was used as the outcome, individuals requiring MV who did not have ALI were included in the no ALI group.) To analyze the contribution of the grouped high-risk haplotypes or alleles, covariates considered were the presence of one or two copies of high-risk haplotypes or alleles, age, gender, asthma, history of bronchopulmonary dysplasia, neurological disorder (defined as developmental delay and/or history of seizures), and sickle cell anemia (African Americans). As there was a gap in age in African Americans for this group, age was categorized into two groups for the analysis, <11 yrs and ≥11 yrs. The final multivariable model was built using a manual stepwise approach. We fit a univariate model with each variable and kept covariates with a $p < .2$, and then we fit models with two of the covariates retained from the first step with their interaction and retained these variables if $p < .2$. Combinations of three variables were then studied of those retained from the previous step. Variables and two-way interactions were studied, including the interaction of a haplotype to a clinical or demographic variable and two clinical or demographic variables.

The indicated p values for the univariate analyses have not been adjusted for multiple comparisons. There is controversy as to whether the adjustment is necessary for studies aimed at examining genetic variations with functional impact on a protein that has biologic relevance to the disease process, as described in this study. However, adjusting the univariate analyses for multiple comparisons using the Bonferroni correction (the most stringent approach), significance is defined in this study as $p < .0125$ (.05/4; analysis of high-risk haplotypes and three individual genetic variants).

RESULTS

General characteristics of the cohort of Caucasian or African American children with CAP are shown in Table 1. Samples were genotyped for the sites of interest. Allele frequencies for the p.508del site (Caucasians, 0.025; African Americans: p.508del, 0.001) and the M470V site (Caucasians, M470, 0.44; African Americans, M470, 0.88) were similar to those previously reported (47). Frequencies of the $(TG)_mT_n$ alleles in the Caucasian children with pneumonia (Table 2) are similar to those previously reported for healthy Caucasians (48, 49). Frequencies in African American children with pneumonia are not significantly different ($p = .23$) than those observed in healthy African Americans (Table 2). LD between the $(TG)_mT_n$ site and the other two sites was examined as described under Methods. The $(TG)_mT_n$ site was in significant LD with the M470V site in both Caucasian Δ' ($D' = 0.53$; $r^2 = .13$; $p < .001$) and African American (Delta prime = 0.67; $r^2 = .06$; $p < .001$) children with CAP. There was no significant LD between the $(TG)_mT_n$ site and the p.508del site in African Americans, although there was in Caucasians (Delta prime = 0.57; $r^2 = .02$; $p < .001$).

Haplotype frequencies in children genotyped at all three sites are shown in Table 3. In Caucasians, haplotypes were classified *a priori* as being high risk if they included the p.508del mutation or a variant at the $(TG)_mT_n$ site (TG≥12 and/or T≤5), which affected the level of functional CFTR (22–26). As there was only one copy of the p.508del mutation in the African American cohort, only $(TG)_mT_n$ alleles were used to determine high-risk variants. The M470V site was not used to assign high-risk haplotypes in either Caucasians or African Americans as this site was found to be in significant LD with the $(TG)_mT_n$ site in both cohorts and because, as discussed in the Introduction, there is no evidence that different alleles at the M470V site have different activity *in vivo*.

As shown in Table 4, in African American children, high-risk $(TG)_mT_n$ alleles (as indicated by the alleles shaded in Table 2) were significantly associated with MV ($p = .0013$) when those with one or two high-risk alleles were compared to those with only low-risk alleles. Trend analysis indicated that the use of MV increased with the number of high-risk alleles with 7% (23/353) of children with two low-risk alleles, 15% (16/105) of those with one high-risk allele, and 36% (4/11) of those with two high-risk alleles requiring MV ($p < .0005$). Caucasian children with CAP requiring MV tended to have more individuals with one or more high-risk haplotypes; however, this association did not reach significance. As shown in Table 5, characteristics of children who required MV are similar to those who did not require MV in both the Caucasian and African American groups.

$(TG)_mT_n$ alleles in African Americans were also significantly associated with ALI when individuals with one or more high-risk alleles were compared to those with two low-risk alleles ($p = .0061$; Table 6). Trend analysis showed that the presence of ALI was correlated to the number of high-risk $(TG)_mT_n$ alleles with 4% (15/353) of those without high-risk alleles having ALI, 10% (10/105) with one high-risk allele having ALI, and 36% (4/11) of those with two high-risk alleles having ALI ($p < .0008$). There was no association of high-risk haplotypes with ALI in Caucasian children.

Association of genotypes at each individual site with lung injury was also examined to determine whether a single site was associated with lung injury. In Caucasian children (Table 7), heterozygous for p.508del 31% required MV, whereas 13% of those without this mutation required MV; however, this difference was not significant ($p = .18$); p.508del was not associated with ALI. Neither the M470V polymorphism nor the $(TG_mT_n$ variable repeat region considered alone was associated with MV or ALI in Caucasian children. In African American children (Table 8), no association of the M470V polymorphism with either MV or ALI was observed.

Multivariable logistic regression analysis was performed to determine whether high-risk haplotypes (Caucasians) or $(TG)_mT_n$ alleles (African Americans) were associated with lung injury after other factors that may influence the severity of disease were considered. Covariates included in the analysis were age, gender, and comorbid conditions. For African American children, multivariable analysis indicated that high-risk $(TG)_mT_n$ alleles were independently associated with both MV and ALI, and this association was highly significant with odds ratios of 3.19 ($p = .0007$) and 3.36 ($p = .0032$), respectively (Table 9). There were no significant interactions. To ensure that subjects who needed MV but did not have ALI were not confounding the ALI results, a multivariable logistic model excluding such patients (n = 14) was also used; the results did not differ from those in Table 9. Multivariable analysis did not show significant association of high-risk haplotypes with the need for MV or ALI in Caucasian children.

DISCUSSION

To our knowledge, this is the first study examining genetic variation in CFTR and lung injury from CAP in children. In African American children with CAP, we observed a strong association of $(TG)_mT_n$ alleles linked to decreased levels of functional CFTR with more severe lung injury. Univariate analysis showed a strong association with MV and ALI with *p* values that were significant even after adjusting for multiple comparisons. In addition, multivariable analyses demonstrated that high-risk $(TG)_mT_n$ alleles were independently associated with MV or ALI with highly significant odds ratios of 3.19 ($p = .0007$) and 3.36 ($p = .0032$), respectively.

The association of specific CFTR $(TG)_mT_n$ alleles with more severe lung injury is likely due to the impact of such alleles on levels of functional CFTR and to the role of CFTR in cyclic adenosine monophosphate up-regulated fluid clearance (6–8) and in regulation of the inflammatory response (15, 16, 19). Interestingly, both the p.508del mutation and exon 9 skipping, which occurs much more frequently with high-risk $(TG)_mT_n$ alleles, generate a CFTR protein product with changes in the first nucleotide-binding domain that fails to traffic to the cell membrane (20, 21); like the p.508del mutant CFTR protein, CFTR without exon 9 is retained in the endoplasmic reticulum and degraded (20, 29, 30). Cells without CFTR, or with p.508del mutant CFTR, have an elevated inflammatory response compared to cells with the wild-type protein (13, 15–19); however, the effect of high-risk $(TG)_mT_n$ alleles on the inflammatory response has not been examined. Data indicate that mutated CFTR may modulate inflammation in cases where misfolded CFTR accumulates in the endoplasmic reticulum, in part by triggering increased activation of nuclear factor–kB and

release of pro-inflammatory cytokines (17). Reports also indicate that normal wild-type CFTR suppresses NF-kB signaling and acts as a negative modulator of inflammation (13, 16). Interestingly, in a mouse model, the lack of functional CFTR on neutrophils enhances the development of ALI triggered by lipopolysaccharide derived from Gram-negative bacteria (19). Together, these findings suggest that CFTR may be important not only for fluid clearance in the lungs but to maintain a balanced inflammatory response. Unfortunately, the clinical data available for this cohort of children do not include information that might indicate whether altered fluid clearance or altered immune response is present in African American children with CAP who are heterozygous or homozygous for high-risk $(TG)_mT_n$ alleles. Future studies will have to address this question.

It is unclear why the association of high-risk $(TG)_mT_n$ alleles with lung injury is observed in African American children but not Caucasian children. The frequency of high-risk $(TG)_mT_n$ alleles is comparable in African American and Caucasian children with pneumonia (0.135 and 0.165, respectively; Table 2). In addition, $(TG)_{12}T_7$ and $(TG)_{11}T_5$ are the most common high-risk alleles in both populations. One possible explanation for this lack of association in Caucasian children may be that there are differences in the level of exon 9 skipping between races in individuals with the same $(TG)_mT_n$ alleles. Studies demonstrating that exon 9 skipping is higher in primary epithelial cells with a higher number of TG repeats and/ or a lower number of thymidines at the $(TG)_mT_n$ site do not indicate the race or ethnicity of the individuals who were the source of the samples. However, there may be underlying differences between African Americans and Caucasians in the surrounding genomic structure that modulate the degree of exon 9 skipping. A number of reports have demonstrated that variations in sequences at sites near exon 9 and the $(TG)_mT_n$ site can modulate the degree of exon 9 skipping with a given $(TG)_mT_n$ allele and hence the amount of functional CFTR protein (25, 50). Such sites affect binding of splicing regulatory factors (25, 27, 28, 51, 52). Another possibility is that the intracellular level of specific regulatory splicing factors differs between Caucasians and African Americans resulting in a difference in the degree of exon 9 skipping with identical $(TG)_mT_n$ alleles. There are *in vitro* data indicating that modulation of the amount of TDP-43, a splicing protein known to be involved in regulation of exon 9 skipping, affects the amount of exon 9 skipping (53). In addition, TDP-43 levels vary across tissue types (54). Alternatively, there may be other differences between Caucasians and African Americans that either affect exon 9 skipping or allow Caucasians to better compensate for less functional CFTR protein.

The finding that African American children with CAP who also had asthma appeared protected from the need for MV was somewhat surprising. However, the *p* value was modest ($p = .04$) and the effect was not significant with ALI as the outcome. Consequently, it is possible that as the use of MV was at the discretion of the treating physician, other therapies might have been used in children with asthma who presented with CAP. The use of other therapies may have influenced the number of asthmatics who were treated with MV.

This study is the first to examine the $(TG)_mT_n$ site in healthy individuals of African descent. Previous reports on individuals of African descent have characterized the number of thymidines and/or the TG repeat region separately and have either examined a relatively small number of individuals or have examined this region in individuals with disease or their

family members (49, 55, 56). Our results show that eight of the nine alleles identified in Caucasians are also present in African Americans. There are a number of additional alleles found at a very low frequency unique to the individuals of African descent.

One limitation of this study is that the amount of functional CFTR in each patient was not measured (only DNA samples were available). Rather, identification of genotypes or haplotypes resulting in decreased functional CFTR was based on previous studies, which demonstrated both in patient samples (22–24) and using *in vitro* systems (25, 26) that specific alleles at the indicated sites (p.508del or $(TG)_mT_n$) were associated with decreased levels of functional CFTR. The choice of specific variants as high-risk variants is also supported by association of these specific sites with disease. The p.508del mutation is the most common cause of CF (57). Specific alleles at the $(TG)_mT_n$ site are associated with congenital bilateral absence of the vas deferens (24) and can cause atypical CF when found in conjunction with a known CFTR mutation (48). Interestingly, two high-risk $(TG)_mT_n$ alleles have not been reported to cause CF. However, the $(TG)_mT_n$ alleles assigned as high risk in this study have been reported to have anywhere from 30% to 90% skipping of exon 9 (24, 29) and consequently have 10–70% of normal CFTR activity. As symptoms associated with CF are thought to occur with loss of ≥95% of CFTR activity (12) even in individuals homozygous for high-risk $(TG)_mT_n$ alleles, there is still sufficient CFTR activity such that individuals do not present with CF.

Another limitation of this study is that only the most common variants reported to affect function were used to generate haplotypes (minor allele frequencies ranged from 0.025 to 0.44), and it is possible that some of these patients have very rare variants (such as one of the >1,500 CF causing mutations, see (58)) or previously unidentified variants, which affect function. The number of children with lung injury as indicated by need for MV, or ALI, is still relatively small (n = 42 and 32, respectively, in Caucasians and n = 43 and 29 in African Americans). Consequently, this study was only powered to observe associations of the grouped high-risk haplotypes or alleles, giving minimal detectable odds ratios for the need for MV of 2.2 for Caucasian and African American children, and minimal detectable odds ratio for ALI of 2.35 for Caucasian and 2.45 for African American children. Associations with lesser effects may have been missed. The limited number of children needing MV or developing ALI also impacts the multivariable analyses, and it is possible that additional factors may have been independently associated with lung injury if a larger population was examined. Furthermore, the two primary outcome measures evaluated in this study (MV and ALI) are not independent. All the children with ALI in this cohort were mechanically ventilated. The need for MV was chosen as an outcome that expanded the degree of lung injury to include children with substantial lung injury that did not meet ALI criteria. However, the need for MV was not explicitly defined and was at the discretion of the attending physician. Last, the findings reported here need to be replicated in another African American cohort; however, currently there are no other pediatric cohorts that have been used to examine lung injury and no African American adult cohorts which have been used to examine pneumonia-induced lung injury. Consequently, our findings should be viewed with caution pending future replication in an independent sample. The National Cancer Institute-National Human Genome Research Institute Working Group on Replication in Association

Studies has stated that such initial findings still provide valuable information when a study has been carefully designed and described (59).

CONCLUSIONS

In conclusion, this study demonstrates an association of alleles at the $(TG)_mT_n$ site in *CFTR*, with the development of lung injury in African American children with CAP and suggests that CFTR may have a role in lung injury in African American patients. These findings support the possibility that some patients with ALI may benefit by using novel therapies targeting CFTR. Future studies will be required to determine whether the findings reported here are replicated in another African American cohort with pneumonia-induced lung injury, whether the $(TG)_mT_n$ site is associated with CAP-induced lung injury in adults or in other racial or ethnic groups, whether there are other sites in CFTR that are associated with lung injury in Caucasians, and whether genetic variation in CFTR is associated with lung injury triggered by other processes.

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Table 1

Characteristics of African American and Caucasian children with community-acquired pneumonia

	Caucasians	African Americans
Age, yrs, median (range)	4.0 (18 days–17.9 yrs)	2.1 (14 days–18.9 yrs)
Gender, n (%)		
Male	165 (54)	249 (53)
Comorbid conditions, n (%)		
Asthma	46 (15)	97 (20)
Bronchopulmonary dysplasia[a]	6 (2)	10 (2)
Neurological disorders[b]	24 (8)	36 (8)
Sickle cell disease	0 (0)	57 (12)
Identified pathogens[c], n (%)		
Bacterial[d]	56 (18)	40 (8)
Viral[e]	22 (7)	43 (9)
Fungal[f]	4 (1)	5 (1)
Mechanical ventilation, n (%)	42 (14)	43 (9)
Acute lung injury[g], n (%)	32 (11)	29 (6)
Mortality, n (%)	2 (0.7)	3 (0.6)

[a] History of bronchopulmonary dysplasia (none of these subjects were on oxygen therapy at home);

[b] neurological disorders included seizures and/or developmental delay;

[c] some patients had more than one identified pathogen;

[d] bacterial pathogens identified by culture of blood, deep tracheal suctioning sample, or pleural fluid; mycoplasma pneumoniae was identified by positive serology; 253 Caucasians and 416 African Americans were tested;

[e] viral pathogens were identified by direct fluorescent antibody staining or polymerase chain reaction of samples obtained by nasal swab, 89 Caucasians and 113 African Americans were tested;

[f] 253 Caucasians and 416 African Americans were tested.

n = 304 for Caucasian children and 474 for African American children.

Table 2

Frequency of $(TG)_mT_n$ alleles

	Caucasians with Pneumonia	African Americans with Pneumonia	Healthy African Americans
Alleles	**n (Relative Frequency)**		
$(TG)_8T_{11}$	0	1 (0.001)	0
$(TG)_9T_9$	2 (0.003)	6 (0.006)	0
$(TG)_9T_7$	0	1 (0.001)	0
$(TG)_{10}T_9$	52 (0.086)	116 (0.124)	28 (0.122)
$(TG)_{10}T_8$	0	2 (0.002)	1 (0.004)
$(TG)_{10}T_7$	111 (0.183)	429 (0.457)	102 (0.443)
$(TG)_{11}T_9$	12 (0.020)	70 (0.075)	13 (0.057)
$(TG)_{11}T_7$	331 (0.544)	186 (0.198)	61 (0.265)
$(TG)_{11}T_5$	14 (0.023)	43 (0.046)	6 (0.026)
$(TG)_{12}T_7$	79 (0.130)	79 (0.084)	16 (0.070)
$(TG)_{12}T_6$	0	2 (0.002)	0
$(TG)_{12}T_5$	6 (0.010)	3 (0.003)	2 (0.009)
$(TG)_{13}T_7$	1 (0.002)	0	0
$(TG)_{13}T_5$	0	0	1 (0.004)

Caucasians with pneumonia, n = 608 chromosomes, 304 individuals; African Americans with pneumonia, n = 938 chromosomes, 469 individuals (five individuals were not successfully genotyped at this site). Healthy African American adults, n = 230 chromosomes, 115 individuals. High-risk alleles are shaded.

Table 3

Frequency of cystic fibrosis transmembrane conductance regulator haplotypes in children with community-acquired pneumonia

	Caucasian	African American
Haplotype	**n (Relative Frequency)**	
$(TG)_8T_{11}$ –A-WT	0	1 (0.001)
$(TG)_9T_9$ -A-WT	2 (0.003)	6 (0.006)
$(TG)_9T_7$-A-WT	0 (0)	1 (0.001)
$(TG)_{10}T_9$-A-WT	37 (0.061)	115 (0.123)
$(TG)_{10}T_9$-G-WT	4 (0.007)	0
$(TG)_{10}T_9$-A-p.508del	11 (0.018)	1 (0.001)
$(TG)_{10}T_8$-A-WT	0	2 (0.002)
$(TG)_{10}T_7$-A-WT	103 (0.169)	427 (0.455)
$(TG)_{10}T_7$-G-WT	8 (0.013)	2 (0.002)
$(TG)_{11}T_9$-A-WT	12 (0.020)	70 (0.075)
$(TG)_{11}T_7$-G-WT	312 (0.513)	106 (0.113)
$(TG)_{11}T_7$-A-WT	19 (0.031)	80 (0.085)
$(TG)_{11}T_5$-A-WT	14 (0.023)	43 (0.046)
$(TG)_{12}T_7$-A-WT	67 (0.110)	74 (0.079)
$(TG)_{12}T_7$-G-WT	12 (0.020)	5 (0.005)
$(TG)_{12}T_6$-A-WT	0	2 (0.002)
$(TG)_{12}T_5$-A-WT	0	1 (0.002)
$(TG)_{12}T_5$-G-WT	6 (0.010)	2 (0.002)
$(TG)_{13}T_7$-A-WT	1 (0.002)	0

Genotypes at the $(TG)_mT_n$, M470V (A or G), and p.508del (WT or p.508del) sites were used to generate three site haplotypes. High-risk haplotypes are shaded. Caucasians, n = 608 chromosomes, 304 individuals; African Americans, n = 938 chromosomes, 469 individuals.

Table 4

Association of high-risk cystic fibrosis transmembrane conductance regulator variants with the need for mechanical ventilation

	Total	−MV	+MV	
		n (%)		p^a
Caucasians (n = 304)				
1 or 2 high-risk haplotypes[b]	95 (32)	78 (30)	17 (41)	.21
2 low-risk haplotypes	209 (68)	184 (70)	25 (58)	
African Americans (n = 469)				
1 or 2 high-risk $(TG)_mT_n$ alleles	116 (25)	96 (23)	20 (47)	.0013
2 low-risk $(TG)_mT_n$ alleles	353 (75)	330 (77)	23 (53)	

MV, mechanical ventilation.

[a] Fisher's exact test;

[b] high-risk haplotypes were defined as described under Results. Five individuals were not successfully genotyped for the $(TG)_mT_n$ site, consequently n = 469 for African Americans.

Table 5

Comparison of general characteristics of children with and without mechanical ventilation

Characteristics	−MV	+MV	p[a]
Caucasians, n	262	42	
Age, yrs, median (range)	3.7 (18 days–17.9 yrs)	7.9 (28 days–17.3 yrs)	.003
Male, n (%)	144 (55)	21 (49)	.62
Comorbidities[b], n (%)			
Asthma	41 (16)	5 (12)	.65
Bronchopulmonary dysplasia	6 (2)	0 (0)	>.99
Neurologic disorders	15 (6)	9 (21)	.002
African Americans, n	431	43	
Age, yrs, median (range)	2.2 (14 days–18.9 yrs)	1.4 (25 days–17.9 yrs)	.64
Male, n (%)	223 (52)	26 (60)	.34
Comorbidities, n (%)			
Asthma	94 (22)	3 (7)	.018
Bronchopulmonary dysplasia	7 (2)	3 (7)	.054
Neurologic disorders	30 (6)	6 (14)	.12
Sickle cell disease	54 (13)	3 (7)	.46

[a] Determined by a nonparametric Mann-Whitney test (for age) or Fisher's exact test (for all others);

[b] comorbid conditions were defined as described in Table 1 and under Methods.

Table 6

Association of high-risk cystic fibrosis transmembrane conductance regulator variants with acute lung injury

	Total	−ALI	+ALI	
		n (%)		p^a
Caucasians (n = 304)				
1 or 2 high-risk haplotypes[b]	95 (31)	86 (32)	9 (28)	.84
2 low-risk haplotypes	209 (69)	186 (68)	23 (72)	
African Americans (n = 469)				
1 or 2 high-risk $(TG)_mT_n$ alleles	116 (25)	102 (23)	14 (48)	.0061
2 low-risk $(TG)_mT_n$ alleles	353 (75)	338 (77)	15 (52)	

ALI, acute lung injury.

[a] Fisher's exact test;

[b] high-risk haplotypes were defined as described under Results. Five individuals were not successfully genotyped for the $(TG)_mT_n$ site, consequently n = 469 for African Americans.

Table 7

Cystic fibrosis transmembrane conductance regulator variant genotype frequency in Caucasian children with and without lung injury

	−MV	+MV		−ALI	+ALI	
Variant	**n (%)**		***p*[a]**	**n (%)**		***p*[a]**
Genotype						
p.508del						
p.508del/p.508del	0	0	.18	0	0	>.99
p.508del/WT	8 (3)	3 (7)		10 (4)	1 (3)	
WT/WT	254 (97)	39 (93)		262 (96)	31 (97)	
M470V (A/G)						
GG	83 (32)	13 (31)	.70	84 (31)	12 (38)	.75
AG	127 (48)	23 (55)		135 (50)	15 (47)	
AA	52 (20)	6 (14)		53 (19)	5 (16)	
$(TG)_mT_n$[b]						
High/high	9 (3)	2 (5)	.36	11 (4)	0 (0)	.68
Low/high	65 (25)	13 (17)		70 (26)	8 (25)	
Low/low	188 (72)	27 (64)		191 (70)	24 (75)	

WT, wild type; MV, mechanical ventilation; ALI, acute lung injury.

[a] Fisher's exact test was used for analysis comparing heterozygotes to homozygotes (p.508del), individuals with GG or AG to those with AA (M470V), or comparing one or more high-risk alleles to those with two low-risk alleles ($(TG)_mT_n$);

[b] high-risk alleles are defined as described under Methods and include the following alleles in the Caucasian cohort: $(TG)_{11}T_5$, $(TG)_{12}T_7$, $(TG)_{12}T_5$, and $(TG)_{13}T_7$; n = 304.

Table 8

Cystic fibrosis transmembrane conductance regulator variant genotype frequency in African American children with and without lung injury

	−MV	+MV		−ALI	+ALI	
Variant	**n (%)**		***p*[a]**	**n (%)**		***p*[a]**
Genotype						
M470V (A/G)						
GG	7 (1.6)	0	>.99	7 (1.6)	0	.65
AG	94 (22)	10 (23)		96 (22)	8 (28)	
AA	330 (77)	33 (77)		342 (77)	21 (72)	
$(TG)_mT_n$[b]						
High/high	7 (1.6)	4 (9)	.0013	7 (1.6)	4 (9)	.0061
High/low	89 (21)	16 (37)		95 (22)	10 (34)	
Low/low	330 (77)	23 (54)		338 (77)	15 (52)	

MV, mechanical ventilation; ALI, acute lung injury.

[a] Fisher's exact test was used for analysis comparing individuals with GG or AG to those with AA (M470V) or one or more high-risk alleles to those with two low-risk alleles ($(TG)_mT_n$);

[b] high-risk alleles are defined as described under Methods and include the following alleles in the African American cohort: $(TG)_{11}T_5$, $(TG)_{12}T_7$, $(TG)_{12}T_6$, and $(TG)_{12}T_5$. Five individuals were not successfully genotyped for the $(TG)_mT_n$ site, consequently n = 469 for genotyping at this site; n = 474 at the M470V site.

Table 9

Multivariable analysis of high-risk $(TG)_mT_n$ alleles and risk of lung injury in African American children with community-acquired pneumonia

Variable	Odds Ratio	95% Confidence Interval	*p*
Mechanical ventilation			
High-risk $(TG)_mT_n$ alleles[a]	3.19	1.63–6.26	.0007
Asthma	0.28	0.08–0.94	.04
Age	5.85	2.73–12.53	<.0001
Acute lung injury			
High-risk $(TG)_mT_n$ alleles[a]	3.36	1.50–7.53	.0032
Asthma	0.47	0.14–1.66	.24
Age	8.36	3.59–19.47	<.0001

[a] Children with one or more high-risk $(TG)_mT_n$ alleles compared to children with no high-risk $(TG)_mT_n$ alleles; n = 469.