

## A CYSTIC FIBROSIS MUTATION ASSOCIATED WITH MILD LUNG DISEASE

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**Abstract Background.** Cystic fibrosis is the most common lethal autosomal recessive disorder among whites. Among Dutch patients with cystic fibrosis,  $\Delta F508$  is the most common mutation and  $A455E$  the second most common mutation of the cystic fibrosis transmembrane conductance regulator gene on chromosome 7.  $A455E$  is associated with preserved pancreatic function and residual secretion of chloride across membranes. We investigated whether it is also associated with less severe pulmonary disease in patients with cystic fibrosis.

**Methods.** A total of 33 patients with compound heterozygosity for the  $A455E$  mutation were matched according to age and sex with patients who were homozygous for the  $\Delta F508$  mutation. The pairs were analyzed with respect to the following outcome variables: age at diagnosis, pulmonary-function values, and the frequency of *Pseudomonas* colonization, pancreatic sufficiency, and diabetes mellitus.

**Results.** Cystic fibrosis was diagnosed at a later age in the patients with the  $A455E$  mutation than in the  $\Delta F508$  homozygotes (mean age at diagnosis, 15.0 vs. 3.1 years;  $P < 0.001$ ). Fewer patients with the  $A455E$  mu-

tation had pancreatic insufficiency (21.2 percent vs. 93.9 percent,  $P < 0.001$ ), and none had diabetes mellitus (0 percent vs. 27.3 percent,  $P = 0.004$ ). Forced expiratory volume in one second (FEV<sub>1</sub>) and forced vital capacity (FVC) were significantly higher in the patients with the  $A455E$  mutation (mean FEV<sub>1</sub>, 73.9 percent of the predicted value vs. 54.3 percent of the predicted value;  $P = 0.002$ ; mean FVC, 88.7 percent of the predicted value vs. 76.3 percent of the predicted value;  $P = 0.04$ ). Fewer patients with the  $A455E$  mutation were colonized with *Pseudomonas aeruginosa* (33.3 percent vs. 60.6 percent,  $P = 0.02$ ).

**Conclusions.**  $A455E$  is a common mutation causing cystic fibrosis in the Netherlands. Although several mutations are known to be associated with less severe pancreatic disease, our findings demonstrate a correlation between the  $A455E$  mutation and mild pulmonary disease. Because mortality in this disease depends primarily on the progression of pulmonary disease, patients with the  $A455E$  mutation have a better prognosis than patients who are homozygous for the  $\Delta F508$  mutation. (N Engl J Med 1995;333:95-9.)

CYSTIC FIBROSIS is the most common lethal autosomal recessive disorder among whites, with an incidence of about 1 in 2500 live births. One person in 25 is an asymptomatic carrier.<sup>1</sup> The gene containing mutations responsible for cystic fibrosis was cloned in 1989.<sup>2-4</sup> It codes for the cystic fibrosis transmembrane conductance regulator (CFTR), a chloride channel dependent on cyclic AMP (cAMP). In cystic fibrosis, defective chloride transport across membranes causes a lack of water in external secretions. This leads to tenacious mucus in the lungs and protein plugs in the pancreas and to the characteristically high sweat chloride levels. The cardinal features of the disease are chronic pulmonary infection and exocrine pancreatic insufficiency. The median survival in the Netherlands is currently 27 years (Dutch Cystic Fibrosis Registry: unpublished data), with most patients dying of pulmonary complications.

Since the gene for cystic fibrosis was cloned, there have been several studies on associations between the genotype and the phenotype in cystic fibrosis.<sup>5-8</sup> A number of mutations (*R117H*, *R334W*, *R347P*, *A455E*, and *P574H*) appear to be associated with pancreatic sufficiency<sup>9</sup> and residual transmembrane transport of

chloride.<sup>10,11</sup> The most common mutation,  $\Delta F508$ , is associated with pancreatic insufficiency and severe pulmonary disease.<sup>5,6</sup> There is great variation in the severity of lung disease, but until now no mutation associated with mild pulmonary disease has been found.

Recently, we noted that a group of Dutch patients with cystic fibrosis who carried the  $A455E$  mutation had significantly better lung function than patients homozygous for the  $\Delta F508$  mutation.<sup>12</sup> An association between this mutation and exocrine pancreatic sufficiency has already been described.<sup>9,11</sup> In addition,  $A455E$  appears to be associated with residual secretion of chloride in electrophysiologic studies of rectal-biopsy specimens.<sup>11</sup> Among our patients the  $A455E$  mutation is relatively common and is associated with an older age at diagnosis.<sup>11,13</sup>

Because of the older age at diagnosis and the mutation-dependent residual chloride secretion associated with  $A455E$ , we hypothesized that the presence of this mutation could result in milder lung disease. To investigate this possibility, we compared clinical data from a group of patients with cystic fibrosis carrying this mutation with patients homozygous for the  $\Delta F508$  mutation who were matched according to age and sex.

## METHODS

### Patients

A total of 125 adult ( $\geq 18$  years of age) patients with cystic fibrosis from the Leyenburg cystic fibrosis center in The Hague, 108 children from Sophia Children's Hospital in Rotterdam, and 45 adult patients from Dijkzigt University Hospital in Rotterdam were

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screened for *CFTR* mutations. These patients represent approximately 30 percent of all known patients with cystic fibrosis in the Netherlands. Among the patients screened, 151 were found to be homozygous for the  $\Delta F508$  mutation and 39 were found to have compound heterozygosity for the *A455E* mutation. In the *A455E* compound heterozygotes, the following mutations were found on the other allele:  $\Delta F508$  (27 patients), *1717-IG*→*A* (4 patients), *E60X* (4 patients), *G542X* (2 patients), *R553X* (1 patient), and an unknown mutation (1 patient). The patient with the unknown mutation was excluded from further analysis. The other mutations found in *A455E* heterozygotes are all associated with pancreatic insufficiency and have been classified as severe cystic fibrosis mutations,<sup>8</sup> predicted to produce no functioning *CFTR*.<sup>14</sup> Therefore, all patients with an *A455E* allele were analyzed together. One patient, whose genotype was *A455E*/ $\Delta F508$ , has been described elsewhere.<sup>15</sup> This patient died at the age of 71 and could not be matched with a  $\Delta F508$  homozygote because of her advanced age. No other data on deceased *A455E* compound heterozygotes were available, and therefore no data on deceased patients were included in the analysis of matched pairs.

DNA analysis was performed at the University of Groningen and at Dijkzigt University Hospital. Analysis for the  $\Delta F508$  mutation was carried out by direct polyacrylamide-gel electrophoresis of the *CFTR* exon 10 product of the polymerase chain reaction<sup>16</sup> or as part of a multiplex amplification refractory mutation system.<sup>17</sup> For the analysis of the *A455E* mutation, a specific amplification refractory mutation system was developed (Scheffer H, et al.: unpublished data).

### Demographic Data

Demographic data included each patient's date of birth, sex, and race and ethnic origin. For *A455E* compound heterozygotes, the place of birth was recorded, as were the birthplaces and family names of their parents and grandparents, as far as could be ascertained.

### Clinical Evaluation

General outcome variables included age at diagnosis and height and weight at last clinic visit. Height percentiles for age were calculated with the use of reference values for Dutch children.<sup>18</sup> Weight-for-height percentiles were calculated with the same reference values.

The results of the most recent representative pulmonary-function test (i.e., one not performed during an acute pulmonary exacerbation) were considered. Values for forced vital capacity (FVC) and forced expiratory volume in one second (FEV<sub>1</sub>) were compared with reference values for the European Union, as endorsed by the European Respiratory Society.<sup>19</sup>

We considered patients to be colonized with *Pseudomonas aeruginosa* if a minimum of three consecutive sputum cultures obtained over a period of at least six months grew this microorganism.

The patients were considered to have pancreatic sufficiency if fecal fat excretion was normal (<10 percent) during three-day fat-balance studies or if they had a normal *N*-benzoyl-L-tyrosyl-*p*-aminobenzoic acid test,<sup>20</sup> normal fecal chymotrypsin concentrations, or normal serum  $\beta$ -carotene concentrations when not using pancreatic-enzyme supplements. Patients with abnormal test results were considered to have pancreatic insufficiency.

Diabetes mellitus was considered to be present when insulin injections were needed to control blood glucose levels.

### Statistical Analysis

Each of the patients with the *A455E* mutation was matched with the  $\Delta F508$  homozygote closest in age, within two years, and of the same sex. The patient who died at the age of 71 and four other *A455E* compound heterozygotes (40, 43, 53, and 47 years old, with the first three having a genotype of *A455E*/*1717-IG*→*A* and the fourth a genotype of *A455E*/ $\Delta F508$ ) could not be matched within two years of age with a  $\Delta F508$  homozygote and were excluded. The  $\Delta F508$  homozygotes who were entered in the matched-pair analysis were compared with the whole group of  $\Delta F508$  homozygotes to determine whether their pulmonary function was representative of that of the group as a whole. In the matched-pair analysis, continuous variables were compared by a two-tailed paired t-test and categorical variables

Table 1. Characteristics of Pairs of  $\Delta F508$  Homozygotes and *A455E* Compound Heterozygotes Matched According to Sex and Age.\*

CHARACTERISTIC	NO. OF PAIRS	$\Delta F508$ HOMOZYGOTES	<i>A455E</i> COMPOUND HETEROZYGOTES†	P VALUE
Sex — no. (%)	33			
Male		16 (49)	16 (49)	NS
Female		17 (51)	17 (51)	
Age — yr	33			
Mean		22.9	23.0	NS
Range		0–40	1–41	
Age at diagnosis — yr	33	3.1±3.9	15.0±10.6	<0.001‡
FEV <sub>1</sub> — % of predicted value	29§	54.3±28.4	73.9±25.5	0.002‡
FVC — % of predicted value	29§	76.3±24.4	88.7±21.1	0.04‡
<i>Pseudomonas</i> colonization — no. (%)	33	20 (60.6)	11 (33.3)	0.02¶
Pancreatic insufficiency — no. (%)	33	31 (93.9)	7 (21.2)	<0.001¶
Diabetes mellitus — no. (%)	33	9 (27.3)	0	0.004¶
Weight — (percentile)	33	61.0±29.4	53.4±30.3	NS
Height — (percentile)				
Men	16	21.4±25.0	42.0±27.6	0.03‡
Women	17	38.1±28.2	38.7±29.4	NS

\*Plus-minus values are means ±SD. NS denotes not significant.

†The following genotypes were identified: *A455E*/ $\Delta F508$  (25 patients), *A455E*/*E60X* (4), *A455E*/*G542X* (2), *A455E*/*R553X* (1), and *A455E*/*1717-IG*→*A* (1).

‡By two-tailed paired t-test.

§Four patients were not old enough for pulmonary-function testing.

¶By two-tailed exact binomial test.

||Weight was adjusted for height.

were compared by computing exact binomial probabilities. To show the progression of lung disease, a regression line for FEV<sub>1</sub> was calculated for all patients in both groups according to age. The slopes of the two regression lines were compared with an analysis-of-covariance model. All P values were two-tailed, and probabilities of less than 0.05 were considered significant. Data were analyzed with the SPSS statistical package.

### RESULTS

All of the patients were white. One patient (a  $\Delta F508$  homozygote) was of Polish descent, and all of the others were Dutch. As far as could be determined, the families of the *A455E* compound heterozygotes were not related, nor did they come from geographically isolated regions. No other connecting feature, such as religion, could be found among these families. At least four *A455E* heterozygotes were former cigarette smokers, whereas none of the  $\Delta F508$  homozygotes had ever smoked.

Table 1 summarizes the results of the matched-pair analysis. Twenty-nine of the patients were matched within one year of age (88 percent), and the remaining four patients were matched within two years of age. The patients with the *A455E* mutation had significantly better lung function than did the  $\Delta F508$  homozygotes (mean FEV<sub>1</sub>, 73.9 percent of the predicted value vs. 54.3 percent of the predicted value; P=0.002). The mean FVC was 88.7 percent for *A455E* compound heterozygotes and 76.3 percent for  $\Delta F508$  homozygotes (P=0.04). Colonization with *P. aeruginosa* (P=0.02) and pancreatic insufficiency (P<0.001) were significantly

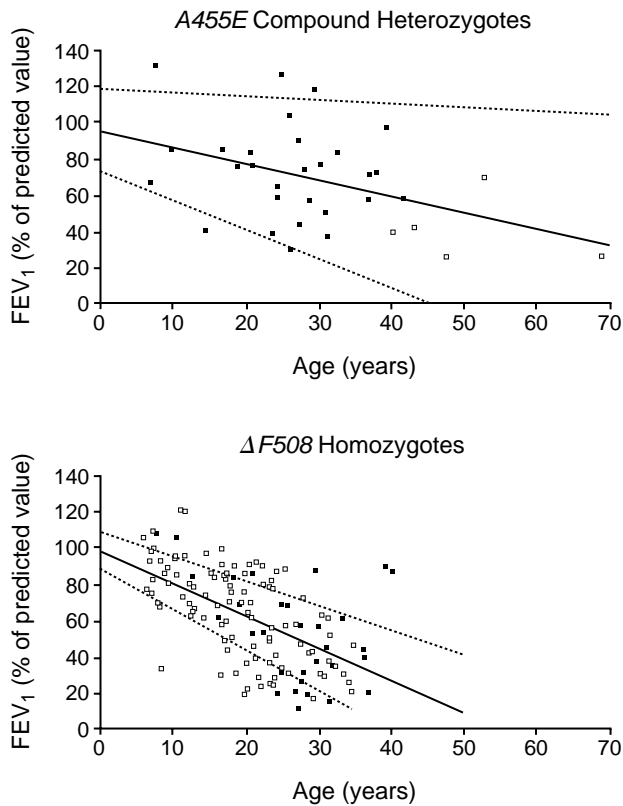


Figure 1. FEV<sub>1</sub> in 34 *A455E* Compound Heterozygotes and 130  $\Delta F508$  Homozygotes, According to Age.

Solid squares represent patients included in the matched-pairs analysis; 4 *A455E* compound heterozygotes and 21  $\Delta F508$  homozygotes were not included because they were too young for pulmonary-function testing. The regression lines (solid lines) and 95 percent confidence intervals (dashed lines) are indicated. The regression line had a slope of  $-0.0089$  for *A455E* compound heterozygotes and  $-0.0178$  for  $\Delta F508$  homozygotes ( $P=0.02$ ).

less prevalent in patients with the *A455E* mutation, and diabetes mellitus was absent in this group ( $P=0.004$ ). Weight (adjusted for height) was normal and not significantly different in the two groups. The men with the *A455E* mutation, but not the women, were significantly taller than matched  $\Delta F508$  homozygotes.

Figure 1 shows the distribution of FEV<sub>1</sub> (as a percentage of the predicted values) for all patients according to age. The regression line had a slope of  $-0.0089$  for *A455E* compound heterozygotes and a significantly steeper slope of  $-0.0178$  for  $\Delta F508$  homozygotes ( $P=0.02$ ). There were no significant differences in lung function between the  $\Delta F508$  homozygotes included in the matched-pair analysis and all other  $\Delta F508$  homozygotes.

**DISCUSSION**

The results of this study show that there is a relation between the presence of the *A455E* mutation and milder lung disease in patients with cystic fibrosis. Although the *A455E* mutation is known to be associated with

pancreatic sufficiency,<sup>9</sup> the association between *A455E* or any other cystic fibrosis mutation and milder lung disease has apparently not been found before.

In earlier studies of the association between genotype and phenotype, large variations in the severity of lung disease among patients with cystic fibrosis and the same genotype have been noted.<sup>8,21,22</sup> Patients who are homozygous for the  $\Delta F508$  mutation have been shown to have more severe lung disease than  $\Delta F508$  heterozygotes with pancreatic sufficiency.<sup>5,6</sup> A number of studies of disease severity in patients with mutations not involving  $\Delta F508$  have been conducted,<sup>23-26</sup> but the results were inconclusive because the numbers of patients were small. To overcome this problem, the Cystic Fibrosis Genotype-Phenotype Consortium initiated a large multicenter study that included 798 patients with cystic fibrosis.<sup>8</sup> Of the eight mutations studied, none were associated with mild lung disease (*A455E* was not included). *R117H*, a mutation associated with residual transmembrane chloride transport,<sup>10</sup> was also associated with an older age at diagnosis and with pancreatic sufficiency, but not with better lung function.

The severity of disease in cystic fibrosis is determined by the mutation resulting in the least severe disease.<sup>9</sup> All the *A455E* compound heterozygotes included in our study had a mutation on their other allele that was associated with severe disease. Mild symptoms in these patients are therefore most likely associated with the presence of the *A455E* mutation. By studying patients seen at two collaborating centers in nearby cities, we minimized the influence of differences in therapy and environmental factors. This is in contrast to the study by the Cystic Fibrosis Genotype-Phenotype Consortium,<sup>8</sup> which included 48 centers in 15 countries.

Table 2. Frequency of  $\Delta F508$  and *A455E* Mutations in Various Countries.

STUDY	COUNTRY/REGION	No. OF CHROMOSOMES SCREENED	%	
			$\Delta F508$	<i>A455E</i>
Present study	The Netherlands	556	73	7.0
Cystic Fibrosis Genetic Analysis Consortium <sup>33</sup>	The Netherlands*	1043	77.1	3.0
Lindner et al. <sup>34</sup>	Southwestern Germany	220	67	0
Cuppens et al. <sup>35</sup>	Belgium	200	72.5	1.0
Super and Schwarz <sup>26</sup>	Northwestern England	1008	82	0
Shrimpton et al. <sup>37</sup>	Scotland	506	72.3	0.5
Claustres et al. <sup>38</sup>	Southern France	262	63	0
Cutting et al. <sup>39</sup>	Baltimore	163	76.1	0.6
Cutting et al., <sup>39</sup> Zielenski et al. <sup>40</sup>	Toronto	1030	68.4	0.2
Rozen et al. <sup>31</sup>	Quebec	84	71	1
Rozen et al. <sup>31</sup>	Saguenay-Lac St. Jean, Quebec	182	58	8
Cutting et al. <sup>39</sup>	United States†	43	37	0
Cutting et al. <sup>39</sup>	Israel‡	94	30	0

\*Includes 31 *A455E* compound heterozygotes from the present study.

†Blacks were studied.

‡Ashkenazi Jews were studied.

Our results show that the type of mutation has a significant impact on pulmonary function in cystic fibrosis. Because of the relatively high frequency of the *A455E* mutation among our patients, we were able to include 33 patients, as compared with the 23 patients with the *R117H* mutation included in the consortium's report.<sup>8</sup> When sufficient numbers of patients are studied, it should be possible to show that other mutations associated with the preservation of pancreatic function and residual transmembrane chloride transport are also associated with milder pulmonary disease.

*A455E* is a missense mutation that leads to a change from alanine to glutamic acid in amino acid residue 455 of the CFTR protein.<sup>27</sup> CFTR is a chloride transporter driven by cAMP, and the *A455E* mutation is situated in the first nucleotide-binding fold, two residues removed from the so-called Walker-A motif, the proposed site of interaction with the phosphoryl moiety of the bound cAMP.<sup>28</sup> The *A455E* mutation may interfere with the binding of cAMP to the CFTR protein. The CFTR protein coded for by the  $\Delta F508$  mutation is subject to defective intracellular processing and does not reach the cell membrane, but is degraded intracellularly.<sup>29</sup> The CFTR protein containing the *A455E* mutation may also be subject to this mechanism, but to a lesser degree.<sup>30</sup> Unlike  $\Delta F508$  homozygotes, patients with cystic fibrosis who have the *A455E* mutation have residual transmembrane chloride transport,<sup>11</sup> a point that increases the likelihood that at least some functioning CFTR protein reaches the cell membrane.

The *A455E* mutation was first found in patients from the Saguenay-Lac St. Jean region in northern Quebec.<sup>27,31</sup> In this formerly isolated community, the incidence of cystic fibrosis and other inherited diseases is higher than normal.<sup>32</sup> The *A455E* mutation is seldom found elsewhere in the world (Table 2),<sup>31,33-40</sup> but it is relatively common in the Netherlands, where it is the second most common cystic fibrosis mutation (3.0 percent of all cystic fibrosis alleles).<sup>33</sup> The two mutations that we studied,  $\Delta F508$  and *A455E*, account for 80.1 percent of the mutations found in Dutch patients with cystic fibrosis. In our study the frequency of *A455E* was 7.0 percent, which may be related to the age distribution of our patients.

In our patients, the presence of the *A455E* mutation was associated with the preservation of both endocrine and exocrine pancreatic function, less frequent colonization with *P. aeruginosa*, and mild lung disease. The regression line for FEV<sub>1</sub> according to age was less steep in *A455E* compound heterozygotes than in  $\Delta F508$  homozygotes, an indication that the progression of pulmonary disease is slower in patients with the *A455E* mutation. *A455E* is the first CFTR mutation for which an association with mild lung disease could be found. Because survival in cystic fibrosis is strongly correlated with the progression of pulmonary disease,<sup>41</sup> we expect that patients with cystic fibrosis who have this mutation will have a better prognosis than patients homozygous for  $\Delta F508$ .

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#### IMAGES IN CLINICAL MEDICINE

Images in Clinical Medicine, a weekly *Journal* feature, presents clinically important visual images, emphasizing those a doctor might encounter in an average day at the office, the emergency department, or the hospital. If you have an original unpublished, high-quality color or black-and-white photograph representing such a typical image that you would like considered for publication, send it with a descriptive legend to Kim Eagle, M.D., University of Michigan Medical Center, Division of Cardiology, 3910 Taubman Center, Box 0366, 1500 East Medical Center Drive, Ann Arbor, MI 48109. For details about the size and labeling of the photographs, the requirements for the legend, and authorship, please contact Dr. Eagle at 313-936-5275 (phone) or 313-936-5256 (fax), or the *New England Journal of Medicine* at [images@edit.nejm.org](mailto:images@edit.nejm.org) (e-mail).