

The pathogenic consequences of a single mutated CFTR gene

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Introductory articles

Δ F508 heterozygosity in cystic fibrosis and susceptibility to asthma

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Background. Cystic fibrosis is a recessive disorder mainly characterized by lung disease. We tested the hypothesis that individuals heterozygous for the common cystic fibrosis Δ F508 mutation are at risk of obstructive pulmonary disease. **Methods.** We studied a cross-sectional sample from the general population of Copenhagen, Denmark, aged 20 years and older. We did spirometry to measure forced expiratory volume in 1s (FEV₁) and forced vital capacity (FVC), and did genotyping on blood samples of 9141 individuals. We asked for information on smoking and other factors that could have contributed to obstructive pulmonary disease. **Findings.** We identified 250 carriers of the Δ F508 mutation (2.7% [95% CI 2.5 to 3.1]). 9% of carriers reported having asthma compared with 6% of non-carriers ($p=0.04$). The odds ratio for asthma in participants heterozygous for Δ F508 mutations was 2.0 (1.2 to 3.5, $p=0.02$). Furthermore, among individuals with airway obstruction, the percentage predicted FEV₁ and FVC were significantly lower in participants heterozygous for Δ F508 than in non-carriers (49 vs 58%, $p=0.004$; and 70 vs 82%, $p<0.001$, respectively), mainly due to an effect in those with self-reported asthma. **Interpretation.** Cystic fibrosis Δ F508 heterozygosity may be over-represented among people with asthma and seems to be associated with decreased pulmonary function in people with airway obstruction who also have asthma. (Lancet 1998;351:1911–3)

Mutations of the cystic fibrosis gene in patients with chronic pancreatitis

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Background. The pancreatic lesions of cystic fibrosis develop in utero and closely resemble those of chronic pancreatitis. Therefore, we hypothesized that mutations of the cystic fibrosis transmembrane conductance regulator (CFTR) gene may be more common than expected among patients with chronic pancreatitis. **Methods.** We studied 134 consecutive patients with chronic pancreatitis (alcohol-related disease in 71, hyperparathyroidism in 2, hypertriglyceridemia in 1, and idiopathic disease in 60). We examined DNA for 22 mutations of the CFTR gene that together account for 95 percent of all mutations in patients with cystic fibrosis in the northwest of England. We also determined the length of the noncoding sequence of thymidines in intron 8, since the shorter the sequence, the lower the proportion of normal CFTR messenger RNA. **Results.** The 94 male and 40 female patients ranged in age from 16 to 86 years. None had a mutation on both copies of the CFTR gene. Eighteen patients (13.4 percent), including 12 without alcoholism, had a CFTR mutation on one chromosome, as compared with a frequency of 5.3 percent among 600 local unrelated partners of persons with a family history of cystic fibrosis ($p<0.001$). A total of 10.4 percent of the patients had the 5T allele in intron 8 (14 of 134), which is twice the expected frequency ($p=0.008$). Four patients were heterozygous for both a CFTR mutation and the 5T allele. Patients with a CFTR mutation were younger than those with no mutations ($p=0.03$). None had the combination of sinopulmonary disease, high sweat electrolyte concentrations, and low nasal potential difference values that are diagnostic of cystic fibrosis. **Conclusions.** Mutations of the CFTR gene and the 5T genotype are associated with chronic pancreatitis. (N Engl J Med 1998;339:645–52)

Cystic fibrosis (CF) is a recessive single gene disorder which requires both copies of the cystic fibrosis gene to be mutated. Currently more than 800 mutations have been detected in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene. Mutations have been found in every exon, as well as in many of the flanking intron sequences, and include missense, nonsense, frameshift and splice site mutations as well as amino acid deletions and substitutions. The major mutation, present in approximately 70% of CF chromosomes worldwide, is a deletion of phenylalanine at position 508 ($\Delta F508$). The relative frequency of the other mutations varies among different populations, but most of them are very rare.

CFTR mutations can be divided into five classes.¹ Class I are nonsense, frameshift, and splice mutations that lead to truncations or absence of *CFTR* protein synthesis. Class II mutations, including $\Delta F508$, are mutations that interfere with correct post-translational processing. The vast majority of mutant protein does not reach the apical membrane, although the protein itself is likely to retain normal function.² Class III mutations such as G551D (glycine to aspartic acid change at position 551) give rise to chloride channels that show a reduced response to cAMP stimulation. Class IV mutations alter the ion selectivity or conductance of *CFTR* chloride channels. Class V mutations interfere with correct transcription or translation, resulting in reduced levels of functional *CFTR*.

A very strong genotype/phenotype correlation has been shown for pancreatic function in patients with CF; 85% of patients suffer from pancreatic insufficiency (PI) while 15% are pancreatic sufficient (PS). Patients with PI generally carry "severe" CF mutations whereas PS is associated with "mild" mutations. As a general rule, mutations classified as nonsense, frameshift, splice-site, or amino acid deletions are "severe" mutations and therefore confer the PI phenotype. However, there is no good correlation between the genotype and the severity of the pulmonary disease, which suggests that the lung phenotype might be modulated by additional genetic or environmental factors.

CF heterozygote carriers are generally disease free. The carrier frequency of *CFTR* mutations in the Caucasian population is 4–5%.³ Founder effects and inbreeding have been shown to account for the high incidence of CF in some populations.⁴ A heterozygote advantage has also been postulated to explain this high incidence of *CFTR* mutations. Increased fertility⁵ and increased resistance to tuberculosis,⁶ cholera,⁷ and typhoid fever⁸ of heterozygote carriers have been suggested and some have been demonstrated in animal models. The papers reviewed here by Dahl *et al* and Sharer *et al* now suggest that CF heterozygotes are over-represented in patients with asthma and chronic idiopathic pancreatitis, respectively.

CF mutations and asthma

Dahl *et al*⁹ suggest a link between $\Delta F508$ heterozygosity and an increased susceptibility to asthma. By studying lung function and the genotype of a cross sectional sample from the general Danish population ($n = 9141$), the authors show that heterozygote carriers of the $\Delta F508$ *CFTR* mutation are over-represented in the Danish population of asthmatics. Further, $\Delta F508$ heterozygosity is linked to decreased pulmonary function in patients with asthma, but not in subjects with chronic obstructive pulmonary disease (COPD). Thus, both

forced expiratory volume in one second (FEV₁) and forced vital capacity (FVC) were significantly lower in asthmatics who carried a $\Delta F508$ mutation than in non-carriers, suggesting that the effect of $\Delta F508$ heterozygosity becomes more/only apparent in combination with other factors involved in the aetiology of asthma. One potential criticism of the study relates to the self-classification of the participants into the presence or absence of respiratory disease, and whether this was asthma or COPD. Further, the authors discuss that a close proximity between the $\Delta F508$ mutation and an unidentified asthma gene (linkage disequilibrium) on chromosome 7 could be an alternative explanation, since samples were drawn only from the Danish population and thus have a high likelihood for common ancestry.

In support of their conclusion, increased bronchial hyperreactivity has previously been demonstrated in one study¹⁰ in heterozygote carriers. Further, an increase in atopic disease in this population has also been documented.¹¹ Dahl *et al* postulate that the increase in bronchial hyperreactivity might explain the association between $\Delta F508$ carriers and asthma. In contrast, a high profile study by Schroeder *et al* suggested that obligate heterozygote carriers ($n = 100$) in CF families in the USA are protected from asthma in childhood and early adult life.¹² This was rapidly followed by a study by Mennie *et al*¹³ who assessed samples from a British population screening programme ($n = 186$) and did not find any association between *CFTR* mutations and asthma, in keeping with previous work which also did not demonstrate such an association.¹⁴

Dahl *et al* evaluated the genetic status of their participants only for the absence or presence of the $\Delta F508$ mutation. However, other genetic variation such as the well documented thymidine tract polymorphism in intron 8 may also be involved. This polymorphism exists as a 9-, 7- or 5-thymidine variant. The 5-thymidine variant (5T) significantly reduces the amount of normal *CFTR* transcript¹⁵ because intron 8 is inefficiently spliced. The 5T variant on one allele, when coupled with a *CFTR* mutation on the second allele, has been associated with congenital bilateral absence of the vas deferens (CBAVD) in infertile males. This condition is the most extensively studied example of "atypical" CF. Thus, in infertile males in whom renal abnormalities were excluded as a cause for CBAVD, *CFTR* mutations are found in 70% of affected males.¹⁶ The combination of the 5T allele on one copy of the *CFTR* gene with a cystic fibrosis mutation on the other allele, or two different mutations on both *CFTR* alleles, is the most common cause of CBAVD.¹⁷ Despite the fact that men with CBAVD usually have none of the other signs of CF such as decreased pulmonary function,¹⁸ pancreatic insufficiency, or altered sweat electrolyte concentration,¹⁹ this disease is classified as atypical CF. It would have been interesting to know whether the 5T variant is over-represented in asthmatic subjects in the population studied by Dahl *et al*. Given the discrepant findings to date, we are not yet persuaded by the proposed link between *CFTR* mutations and asthma.

CF mutations and chronic pancreatitis

Another disease recently associated with mutations in the *CFTR* gene is chronic pancreatitis. A link between these diseases has been suggested because the pancreatic lesions seen in subjects with CF closely resemble those in chronic pancreatitis. The study by Sharer *et al*²⁰

screened 134 patients with chronic pancreatitis for 22 *CFTR* mutations as well as the length of the thymidine tract in intron 8. Together these alterations account for 95% of *CFTR* mutations in north-west England. The frequency of *CFTR* mutations among patients with pancreatitis was about 2.5 times higher and the 5T variant in intron 8 was twice as high as that of the general population. None of the patients had a mutation on both copies of the *CFTR* gene. However, four of the 18 patients with a *CFTR* mutation also had the 5T variant, which was presumed to be on the opposite allele. The patients with the CF mutations were significantly younger at presentation and included a higher proportion of non-smokers. There was no significant difference in the proportion of alcoholics and non-alcoholics with mutations although non-alcoholics had an approximately twofold greater incidence. In addition to the mutation analysis, a number of investigations frequently used in the diagnosis of CF were assessed. These included sinopulmonary evaluation, measurements of basal potential differences in the nasal epithelium, sweat chloride tests, and semen analysis. Based on these parameters a diagnosis of atypical CF could not be confirmed in any of the patients presenting with chronic pancreatitis.

Alcoholism, smoking, and a number of metabolic disorders are major risk factors in the aetiology of chronic pancreatitis; no cause can be identified in the remaining 40% or so of affected patients.²⁰ Interpretation of the data in the present study is complicated by the heterogeneous aetiology of the patients chosen. Pancreatitis was related to alcoholism in 53% whilst 74% of patients were smokers, making it more difficult to determine the relative contribution of *CFTR* mutations. Clearly, the influence of smoking will also be important with regard to the use of lung function tests in the diagnosis of CF lung disease. Thus, obstructive lung function was found in 22% and 25%, respectively, of patients with and without *CFTR* mutations. Similarly, measurements of sweat electrolyte concentrations, the gold standard for diagnosis of CF, are hampered by the knowledge that alcoholism increases sweat electrolyte levels.

Despite these potential confounding factors, the results presented here are in agreement with a recent study which also determined the prevalence of *CFTR* mutations and the 5T polymorphism in patients with chronic idiopathic pancreatitis.²¹ Nine of 27 patients had a *CFTR* mutation or the 5T variant, or both. In three patients both *CFTR* alleles were affected; the genotypes were the two most common in patients with CBAVD. As is the case with CBAVD subjects, none of these patients fulfilled traditional diagnostic criteria for the classic CF phenotype. Unlike the study by Sharer *et al*, this study did not find evidence for an increased frequency of the 5T variant in patients with chronic idiopathic pancreatitis. However, in a recent correspondence Sharer *et al* recognised a mistake in calculating the carrier frequency of the 5T variant and confirmed that the 5T variant is not associated with chronic pancreatitis.²² In our view, given the concordant findings of both these studies with respect to the increased frequency of *CFTR* mutations, there is compelling evidence for a role for *CFTR* mutations in the aetiology of chronic pancreatitis.

Other non-CF diseases associated with CFTR mutations

Apart from asthma and pancreatitis, the frequency of

Table 1 Non-CF diseases associated with CFTR mutations

Congenital bilateral absence of the vas deferens (CBAVD)
Azoospermia
Asthma
Pancreatitis
Bronchiectasis
Allergic bronchopulmonary aspergillosis

CFTR mutations has been determined in a number of other diseases that present with isolated features of the classical CF phenotype (table 1). The prevalence of *CFTR* mutations and the 5T variant in men with CBAVD is well documented and has already been discussed above. *CFTR* mutations have also been implicated in men with azoospermia without CBAVD, suggesting an important role for *CFTR* in spermatogenesis and sperm maturation^{23,24} but not in sperm malformations.²⁵ Miller *et al* found an increased frequency of *CFTR* mutation in patients with allergic bronchopulmonary aspergillosis. However, this study was carried out on a small number of patients (n=11).²⁶ Two studies determined the incidence of *CFTR* mutations in patients with nasal polyposis but neither found an increased frequency of *CFTR* mutations in these populations.^{27,28} Finally, patients with bronchiectasis or COPD have also been screened for *CFTR* mutations. Several studies report evidence for an involvement of *CFTR* in at least some cases of bronchiectasis^{29,30} but not in COPD,^{31,32} in agreement with the findings of Dahl *et al* discussed above.

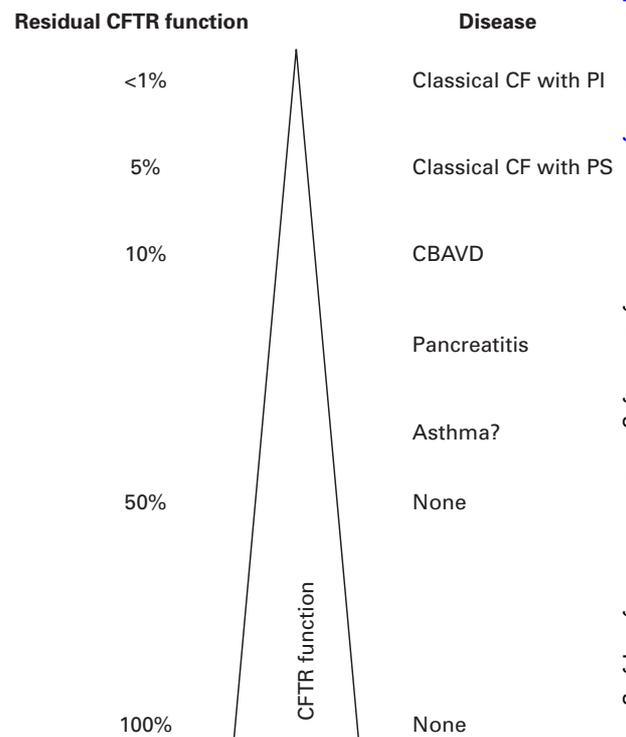


Figure 1 *CFTR* mutations reduce the level of normal *CFTR* function. Disease manifestation depends on the amount of residual *CFTR* function and there appears to be a tissue specific threshold. PS=pancreatic sufficiency; PI=pancreatic insufficiency; CBAVD=congenital absence of the vas deferens.

LEARNING POINTS

- * The pathogenic consequences of mutated *CFTR* can be found in diseases other than classical cystic fibrosis.
- * Chronic pancreatitis appears to be one such disease; the link with asthma is less certain.
- * The presence of the 5T variant in intron 8 should be considered in addition to "classical" CF mutations.

How does a mutation in one *CFTR* allele cause disease?

The relationship between the basic defect in CF (altered transport of chloride (Cl^-) and sodium (Na^+) ions) and the pathology of the disease is not well understood, but for lung disease several hypotheses are available. One simplistic suggestion relates to impaired mucociliary clearance in the airways, since efficient clearance of mucus and inhaled micro-organisms is likely to depend on an optimal volume of airway surface fluid in which the cilia involved in mucociliary clearance beat. Thus, reduced chloride secretion onto, and increased sodium absorption from, the airway lumen may lead to a sub-optimal volume of airway surface liquid and hence impaired mucociliary clearance, increased bacterial colonisation, and repeated infection. However, other defects in host defence may also play a part—for example, increased bacterial adherence,³³ reduced ingestion of bacteria by epithelial cells,³⁴ and impaired antibacterial activity of surface defensins.³⁵ Altered cytokine secretion has recently been detected in CF lungs and might in part explain the chronic inflammation seen in young patients with CF in the absence of bacterial infection.³⁶ The finding that *CFTR* is likely to be involved in the functioning of intracellular processes, particularly endocytosis and exocytosis, may be an important link with disease causation. Thus, if *CFTR* acts at an early stage in the processing of proteins, a wide spectrum of abnormalities could be produced. It is even less well understood how the absence of functional *CFTR* leads to manifestation of the CF phenotype in the other affected organs, but dehydration and mucus accumulation appear to be central to the disease. In addition, impaired *CFTR* mediated bicarbonate (HCO_3^-) secretion has been implicated in causing pancreatic insufficiency in CF. It is generally assumed that heterozygote carriers of *CFTR* mutations have about 50% of normal function, which is sufficient to remain free of disease. However, a further reduction in *CFTR* function due, for example, to the presence of the 5T variant in intron 8 or certain as yet unidentified *CFTR* modifier genes probably causes manifestation of isolated CF disease features such as CBAVD, chronic pancreatitis, and perhaps asthma. The degree of disease manifestation depends partly on the genotype and, in addition, there appears to be a tissue specific minimal requirement for normal *CFTR* function (fig 1). Mild mutations and the 5T variant that result in reduction of *CFTR* function to about 10% lead to CBAVD; mutations that reduce the amount of normal *CFTR* to about 5% cause lung and sweat gland abnormalities, and the most severe mutations (less than 1% of normal *CFTR*) also lead to the pancreatic insufficiency characteristic of CF.

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