Carriers of a single *CFTR* mutation are asymptomatic: an evolving dogma?

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Cystic fibrosis (CF) is a genetic autosomal recessive disease due to mutations in the gene encoding for the cystic fibrosis transmembrane conductance regulator (CFTR) protein [1, 2]. Mutations in the *CFTR* gene may cause a reduction of CFTR protein function, leading to abnormal chloride and bicarbonate transport in epithelia, resulting in abnormal mucus properties and a multiorgan disease dominated by respiratory and gastro-intestinal abnormalities [3]. The level of CFTR protein function is an important determinant of disease in humans and CF patients carrying two disease-causing *CFTR* mutations usually have very low levels of functional CFTR protein. Carriers of a single *CFTR* mutation (also called CF carriers) express 50% CFTR protein function, a level that has been considered sufficient to stay healthy [3]. CF carriers represent approximately 1 in 35 Caucasian in the USA, accounting for more than 10 million individuals [4]. CF carriers are generally informed that they are at risk of transmitting the mutation to their children and/or to have a child with CF; they are also informed that having one *CFTR* gene mutation does not cause symptoms [4]. Having a single *CFTR* mutation has even been suspected to provide a selective advantage, as CF carriers may withstand secretory diarrhoea better than non-carrier individuals, leading to a possible protection against cholera [5]. This latter finding has been suggested to explain the high rate of CF carriers in Caucasian populations [1].

Several studies have reported increased rates of CF carriers in cohorts of patients with bronchiectasis, chronic rhinosinusitis/nasal polyposis, or idiopathic chronic pancreatitis, questioning possible roles of a single *CFTR* mutation in the pathophysiology of these diseases (as reviewed previously [6]). In the present issue of the *European Respiratory Journal*, Çolak *et al.* [7] took the reverse approach: they hypothesised that CF carriers could be at higher risk of death or disease. The authors genotyped 108 035 randomly selected white Danish individuals from the Copenhagen General Population Study for the Phe508del *CFTR* mutation (the most prevalent *CFTR* mutation worldwide) and looked for morbidity and mortality, using questionnaires (for chronic bronchitis), spirometry and medico-administrative databases. The authors found 2858 (3%) carriers of a single Phe508del *CFTR* allele, corresponding to a prevalence of 1 in 38 subjects. Carriers and non-carriers of the Phe508del *CFTR* allele had comparable overall survival. However, carriers of the Phe508del *CFTR* allele had increased risk of chronic bronchitis (multivariable adjusted odds ratio of 1.31), bronchiectasis (multivariable adjusted hazard ratio of 1.88) and lung cancer (multivariable adjusted hazard ratio of 1.52). The authors also found that carriers of the Ph508del *CFTR* allele had non-significant trends for higher risk in chronic rhinosinusitis, spontaneous pneumothorax and

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male infertility (all of which are found in subjects with CF). However, they reported no association for respiratory failure, acute or chronic pancreatitis, liver cirrhosis, gastric or colorectal cancer.

The study by ÇOLAK *et al.* [7] has some limitations: the authors limited the analysis to the most prevalent *CFTR* mutation (Phe508del), presumably in order to reduce the cost of the study. Although it is unlikely that an important number of CF carriers had a second disease-causing *CFTR* mutation (as most patients with CF are diagnosed and followed in Denmark) it appears possible that some people without a Phe508del *CFTR* mutation had another non Phe508del *CFTR* mutation. As these people would have been classified in the non-carrier group, the choice of limiting the analysis to carriers of the Phe508del mutation may have reduced the increased risk of disease attributed to carriers of a single *CFTR* mutation. Further, even in this large cohort study, the statistical significance of the associations could have been affected by the number of patients in the cohort, especially for rare disorders (*e.g.* chronic pancreatitis). The risk of misclassification, due to limitation of the study to Phe508del mutation, and the sample size may have accounted for some surprising results such as the non-significant association with chronic pancreatitis and colorectal cancer. The finding that lung cancer is increased in carrier of a Phe508del mutation is also surprising, because the risk of gastro-intestinal cancer, but not the risk of lung cancer, has been found increased in patients with CF [8]. However, the CF population is still relatively young and has usually low exposure to cigarette smoke, whereas patients in the present study were older and heavily exposed to cigarette smoke. This underlines the importance of controlling risk factors such as cigarette smoke in patients with CF, especially as ageing of the CF population is predicted in many countries [9].

The elegant data by ÇOLAK *et al.* [7] provide an interesting complement to a recently published study by MILLER *et al.* [10], who identified 19 802 CF carriers using a US administrative database and matched each carrier with five controls. The authors reported significantly increased risk for 57 conditions related to CF, including chronic bronchitis and bronchiectasis, male infertility, and pancreatitis [11]. Although there are methodological differences between these studies, which may account for some discrepancies in the results (for example the increased risk of pancreatitis was significant in the study by MILLER *et al.* [10] but not in the present study by ÇOLAK *et al.* [7]), both studies challenge the dogma that CF carriers are asymptomatic. Indeed, both studies reported increased relative risk of multiple diseases in CF carriers compared with non-carriers. Importantly, the absolute risk of disease remained low in both studies [7, 10],

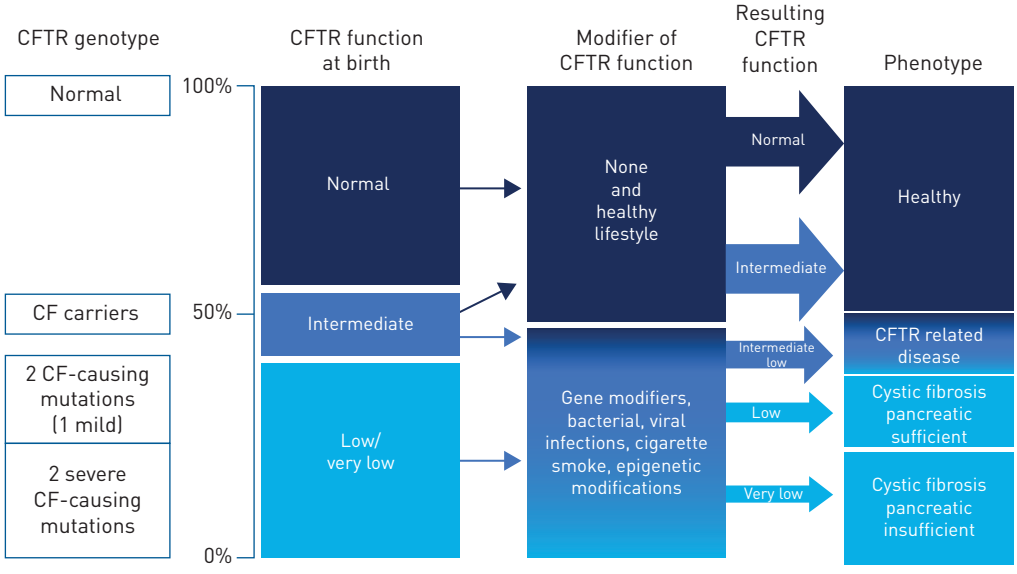

FIGURE 1 Hypothetical relationship between cystic fibrosis transmembrane conductance regulator (CFTR) genotypes and resulting phenotypes. Cystic fibrosis (CF) is a genetic disease resulting from autosomal mutations in the CFTR gene. Patients with two severe disease-causing CFTR mutations have very low CFTR protein function at birth and develop early disease characterised by pancreatic insufficiency and chronic lung disease. Patients with two CFTR mutations, including at least one milder mutation, have low CFTR function at birth and usually develop less severe or delayed respiratory disease with pancreatic sufficiency. Patients with no CFTR mutation (normal genotype) have normal CFTR function and remain healthy when they have a healthy lifestyle. CF carriers, who carry a single CFTR mutation have intermediate (50% of normal) CFTR function at birth and often remain asymptomatic when they have a healthy lifestyle. However, CF carriers are at increased risk of multiple CFTR-related diseases (including chronic bronchitis and bronchiectasis) that may be due to intermediated CFTR function at birth, which may be further reduced in some patients by environmental exposure (cigarette smoke, infection) and/or genetic/epigenetic modifications.

indicating that most CF carriers will indeed remain asymptomatic [10, 12]. For example, only 322/2858 (11.2%) carriers of the Phe508del mutation developed chronic bronchitis in the study by Çolak *et al.* [7]. Even when looking at the risk of multiple diseases, as in the study by Miller *et al.* [10], only a minority of CF carriers developed at least one of these diseases [10].

The studies by Çolak *et al.* [7] and Miller *et al.* [10] provide epidemiological evidence of increased risk of multiple diseases (most often related to respiratory, gastro-intestinal and pancreatic systems) in CF carriers. Interestingly, several studies provide biological plausibility for these findings by linking CF carriers to specific biological abnormalities. Epithelial chloride transport appeared abnormal in CF carriers, as evidenced by higher than normal sweat chloride concentration [13], and by abnormal nasal potential difference measurements in CF carriers with bronchiectasis as compared to patients with bronchiectasis but no *CFTR* mutation [14]. Data obtained in newborn screening programmes have reported higher blood immunoreactive trypsinogen concentrations in CF carriers compared to normal [15, 16], indicating pancreatic abnormalities. Moriceau *et al.* [17] discovered that both children with CF and their parents, who are obligate heterozygotes for *CFTR* mutations, demonstrated delayed neutrophil apoptosis compared with healthy controls. Recent evidence further suggests that CFTR dysfunction could also be acquired: exposure to cigarette smoking could induce CFTR dysfunction in airway epithelium [18] and systemically [19]; viral infection [20], bacterial products [21] and neutrophil proteases [22] could also result in defective CFTR function. Epigenetic CpG island hypermethylation of the *CFTR* gene turns off its transcriptional expression [11]. Thus, it appears possible that CF carriers have reduced CFTR protein function at birth and that environmental exposures/epigenetic regulation may result in further reduction of CFTR protein function, leading to disease in selected patients. It is also possible that multiple gene defects could contribute to disease in CF carriers. For example, it has been suggested that combination of mutations in the epithelial sodium channel (*ENaC*) gene and in the *CFTR* gene predispose to bronchiectasis with abnormal epithelial ion transport [23]. This hypothesis, which is represented in figure 1, would explain why only a subset of CF carriers develop disease, whereas most CF carriers remain asymptomatic.

Findings by Çolak *et al.* [7] and Miller *et al.* [10] clearly change the dogma that CF carriers are always asymptomatic, and these studies may have important consequences. First, the number of people diagnosed as CF carriers will likely continue to increase as genetic testing is now increasingly proposed for cascade screening of a relative of a CF case, for preconception screening in selected populations, and as systematic screening in some geographical areas [24]. Further, the implementation of newborn screening programmes in multiple countries is associated with the detection of eight to ten CF carriers per each diagnosed case of CF [25]. Thus, findings that CF carriers may be at increased risk of diseases represent a challenge for discussing with CF carriers: it may not be any longer appropriate to consider that these patients have no risk of disease, yet most of them will indeed remain asymptomatic. This findings highlight the difficulty in making individual prediction based on the presence of a single mutation in a gene associated with a recessive disorder, but also provide an opportunity for preventative medicine by advising a healthy lifestyle, as the consequences of environmental exposures (*e.g.* cigarette smoking) could prove even more deleterious in CF carriers. Second, the recent development of highly effective CFTR modulators (especially for CF patients with at least one Phe508del allele [26, 27]) provides the opportunity of repositioning CFTR modulators for airway diseases other than CF [28]. The development of CFTR modulator for the treatment of patients with COPD and chronic bronchitis appears of particular interest [29]. The high population attributable risk of bronchiectasis for CF carriers [12] also warrants further interest, as a proportion of patients with non-CF bronchiectasis may derive benefit from CFTR modulators. The findings that loss of normal CFTR activity leads to activation of tyrosine kinase receptors [30] and that CFTR may suppress cancer [31] could also provide a rationale for the repositioning of CFTR modulators for the treatment of cancer (*e.g.* colon cancer [29]).

In summary, the data presented by Çolak *et al.* [7] contribute to further challenging the dogma that CF carriers are strictly asymptomatic. The results provide opportunities for rethinking the relationship of a single *CFTR* mutation to individual risk prediction and strengthens the rationale for repositioning CFTR modulators for the treatment of diseases other than CF. For all these reasons, the authors should be congratulated for their nice report.

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