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Clinical Phenotypes of Cystic Fibrosis Carriers

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Abstract

Cystic fibrosis (CF) is an autosomal recessive genetic disorder caused by mutations in *CFTR*, the cystic fibrosis transmembrane conductance regulator gene. People with CF experience a wide variety of medical conditions that affect the pulmonary, endocrine, gastrointestinal, pancreatic, biliary, and reproductive systems. Traditionally, CF carriers, with one defective copy of *CFTR*, were not thought to be at risk for CF-associated diseases. However, an emerging body of literature suggests that heterozygotes are at increased risk for many of the same conditions as homozygotes. For example, heterozygotes appear to be at increased risk for chronic pancreatitis, atypical mycobacterial infections, and bronchiectasis. In the United States alone, there are almost 10 million CF carriers. Universal newborn screening and prenatal genetic screening will identify more. Thus, there is a critical need to develop more precise estimates of health risks attributable to the CF carrier state across the lifespan.

THE CYSTIC FIBROSIS PHENOTYPE

Cystic fibrosis (CF) is one of the most common autosomal recessive genetic disorders in the United States and the most common among Caucasians of European descent. The disease is caused by mutations in *CFTR*, the cystic fibrosis transmembrane conductance regulator gene, which encodes a chloride and bicarbonate channel expressed in the apical membrane of epithelial cells (1). The gene is expressed in multiple organ systems, which explains the wide variety of medical conditions experienced by people with CF, affecting the pulmonary, endocrine, gastrointestinal, pancreatic, biliary and reproductive systems (2, 3).

People with CF commonly suffer from chronic bronchitis, bronchiectasis, chronic sinusitis, gastroesophageal reflux disease, constipation, diarrhea, diabetes, chronic pancreatitis, malnutrition, delayed development, male infertility, osteoporosis, asthma, and nasal polyposis (4). Other CF-related conditions include fluid and electrolyte disorders, colon cancer, scoliosis, intestinal obstruction, cholelithiasis, and nephrolithiasis (4). All of these conditions contribute to the morbidity associated with CF, but a substantial proportion of CF morbidity is associated with chronic and recurrent respiratory infections. Interestingly, patients with CF are not equally susceptible to all respiratory pathogens. In particular, they are at risk for infections caused by *Pseudomonas*, *Burkholderia*, *Aspergillus*, and nontuberculous mycobacteria (4). People with CF do not appear to be at increased risk from respiratory pathogens associated with other immunocompromised states (e.g., *Pneumocystis jirovecii*). In addition, they do not seem to be at risk for infections outside the respiratory tract. For example, people with CF are not at increased risk for urinary tract or skin-and-soft-tissue infections. Despite the number of antibiotics they are prescribed and their frequent interactions with the healthcare system, people with CF do not appear to be at increased risk for *Clostridioides difficile*–associated diarrhea (5).

Conditions associated with CF are attributed to absent or significantly reduced CFTR function. The mechanistic underpinnings for some CF-related conditions are understood. The fluid and electrolyte disorders associated with CF are one example. People with CF secrete a normal, chloride-rich primary sweat (6, 7), but they fail to reabsorb chloride (and sodium), resulting in substantially higher sodium and chloride concentrations than in the sweat of people without CF (8–10). Thus, excessive sweating in patients with CF, without adequate fluid and electrolyte replacement, can result in profound sodium and chloride depletion (6, 10). The precise mechanisms influencing how absent CFTR function causes other CF-related diseases remain less clear. Indeed, controversy about the basis for persistent respiratory infections continues.

The diversity of clinical phenotypes among people with CF is partially attributed to variants in protein function caused by different *CFTR* mutations (11, 12). In addition, both genetic modifiers and environmental factors alter the clinical phenotype of people with CF. Several genetic modifiers have been identified to date (13–15). Examples of environmental factors include pollution (15–17), the ingestion of sublethal levels of arsenic (18), and cigarette smoke, each of which decreases CFTR function (19, 20). Modifier genes and environmental factors help explain how the same *CFTR* mutations are associated with different clinical presentations.

Generally, mutations (such as the most common, F508del) that cause the most severe disruption of CFTR function are associated with worse clinical outcomes (11, 12, 21). Because heterozygotes carry only one *CFTR* mutation, they express half as many CFTR channels as people without a *CFTR* mutation (22). Historically, the level of CFTR function associated with the CF carrier state was thought to be sufficient for the maintenance of health (23). Thus, when people are informed that they are CF carriers, they are usually told that they are not at increased risk for CF-related disease (24–26). The notion that heterozygotes are not at risk for CF-related disease originates from multiple studies performed in the early 1960s (27–30), but the concept of the healthy

heterozygote state was likely bolstered by the prevailing perception that the CF carrier state contributed a survival advantage, commonly referred to as the heterozygote advantage.

THE HETEROZYGOTE ADVANTAGE

Until recently, most people with CF died early in their reproductive years. Thus, it is surprising how common CF carriers are among some populations. The relatively high frequency of heterozygotes is not likely due to genetic drift or random mutation (31–33). Some early studies supported a fertility advantage hypothesis (34, 35), but these results were most likely due to ascertainment bias, as larger families are more likely to produce cases of recessive diseases. Indeed, two more-recent studies, one in Utah (36) and the other in Hutterites in South Dakota (37), did not find a fertility advantage attributable to the CF carrier state. Instead, the most likely explanation for the relatively high frequency of heterozygotes is that having one copy of a defective *CFTR* gene promotes a survival advantage. This advantage could be subtle and thus difficult to detect, especially because the selective pressure may no longer be present in today's environment. To maintain the current population frequency of heterozygotes, an estimated survival advantage of approximately 2% may be sufficient (34).

The enthusiasm for finding the reason for a CF carrier survival advantage is motivated in part by the example set by another autosomal recessive disease: sickle cell disease. Both people with sickle cell disease and heterozygotes for the sickle cell gene are afforded some protection against malaria (38). Of note, carriers for sickle cell disease are less common in highland areas of Africa where there are fewer mosquitos and, hence, less malaria (39). Because infectious diseases have historically been one of the major drivers of mortality, researchers have proposed that CF carriers may be protected from a range of infectious diseases. For example, investigators have theorized that CF carriers may be protected from severe illness due to influenza (40), syphilis (41), plague (42), malaria (43), and tuberculosis (44, 45). However, none of these examples has a compelling mechanistic explanation for a protective effect. Furthermore, the geographic distribution of these infections does not readily match the historical geographic distribution of CF carrier prevalence. Survival advantages beyond infectious diseases have also been proposed, including respiratory advantages in the dusty climate after the last glaciation period (46, 47) and even the possibility that the CF carrier state is protective against some cancers (48).

The discovery of the cause of CF generated new potential drivers of the heterozygote advantage hypothesis. For example, it was hypothesized that heterozygotes may be resistant to cholera (49). An alternative hypothesis is that heterozygotes may be resistant to dehydration from secretory diarrhea caused by enterotoxigenic *E. coli* (6, 49). Also, some in vitro experiments suggest that *Salmonella typhi*, the cause of typhoid fever, attaches to the intestinal mucosa via CFTR, supporting the notion that heterozygotes may be protected against typhoid fever (50). While the idea is intriguing, the geographic distribution of current and historical CF carriers is not the same as that of typhoid fever or cholera. Despite the many hypotheses proposed, there are no conclusive findings demonstrating any reasons for a survival advantage for CF carriers. In contrast, there is a growing body of evidence suggesting that CF carriers are at risk for not only subclinical laboratory abnormalities but also a range of adverse health outcomes.

SUBCLINICAL DIFFERENCES BETWEEN PEOPLE WITH CYSTIC FIBROSIS AND CARRIERS

To assist with genetic counseling, several early investigations focused on describing subclinical differences between people with CF and obligate CF carriers (the parents of children diagnosed

with CF). There were two major motivations for this early work. First, understanding differences between people with CF and CF carriers might help identify the cause of the disease. Second, measurable differences between people with CF and CF carriers could enable a screening test to identify CF carriers and assist with genetic consulting. Over almost 3 decades of research, several observations have highlighted differences between CF carriers and controls (who were neither people with CF nor CF carriers). For example, with the development of the sweat chloride test, obligate CF carriers were noted to have, on average, higher levels of sweat chloride than controls, but CF carriers had lower levels than people with CF (51). Also, CF carriers and people with CF were noted to have elevated levels of intracellular calcium (52, 53), protein in meconium as infants (54), alpha-fetoprotein (55), and a carcinoembryonic antigen-like substance, compared to controls (56). CF carriers also had abnormal levels of circulating polyamines (57, 58), altered neutrophil function (59), and lower concentrations of both sodium and magnesium in erythrocytes (60). In addition, the beta-adrenergic stimulated secretory response is significantly reduced in CF carriers relative to controls, but not to the extent observed in CF (61), and the blood spot immunoreactive trypsinogen (IRT) assay used in newborn screening programs frequently identifies heterozygotes because they often have higher IRT values than people without *CFTR* mutations (62). Even for infants with an IRT value within the normal range, the probability of being a CF carrier increases with higher IRT levels (63).

Finally, in the late 1960s, several research groups attempted to develop bioassays to detect CF carriers based on the initial observation that sera from both people with CF and CF carriers disrupted the normal ciliary activity in the trachea of rabbits (64). The disruption of ciliary movement by sera from people with CF and CF carriers was replicated in both oysters (65) and freshwater mussels (66). The substance responsible for the disruption of normal ciliary movement was labeled CF dyskinesia substance. Similar factors were also found to be excreted by peripheral blood leukocytes from CF carriers (67). While interesting, none of these experiments produced results sufficiently robust to enable development of a bioassay with sufficient sensitivity and specificity to reliably distinguish people with two defective copies, one defective copy, or no defective copies of *CFTR*. Although these tests were not clinically useful, the experiments highlighted another subclinical difference between heterozygotes and people with two functioning copies of *CFTR*.

CLINICAL DIFFERENCES BETWEEN PEOPLE WITH CYSTIC FIBROSIS AND CARRIERS

The development of the sweat chloride test enabled more objective criteria for identifying cases of CF. Following this innovation, multiple early investigators presented data supporting the notion that CF carriers, the parents of children with CF, were at increased health risk, specifically for respiratory (68–70) and gastrointestinal diseases (71). However, in the early 1960s, four papers from three different countries, Australia (27), England (28), and the United States (29, 30), all concluded that obligate carriers for CF were not at increased health risk. Together, these studies reinforced the notion of the healthy heterozygote, yet all of these studies were relatively small (95–144 obligate heterozygotes) (27–30). Approximately 40 years later, Castellani et al. (23) surveyed the parents of CF patients about the presence of multiple CF-related conditions. The sample for this study was almost double the number of previous studies (including 261 obligate heterozygote parents as well as controls), but the only CF-related condition that was increased in heterozygotes compared to controls was hypertension. While all of the earlier studies strongly dismissed the possibility of a distinct clinically significant phenotype for heterozygotes, one has to

wonder, if these studies were larger, would they have come to a different conclusion? For example, Castellani et al. (23) considered 12 conditions (asthma, bronchiectasis, pneumothorax, allergic bronchopulmonary aspergillosis, sinusitis, nasal polyps, gallstones, liver cirrhosis, diabetes, pancreatitis, bone fractures, and hypertension). Almost all were more common in heterozygotes than controls, and heterozygotes were more likely to have two of the conditions (18 heterozygotes versus 14 controls) or three conditions (9 versus 0).

The introduction of genetic testing for CF enabled investigators to determine if CF carriers were overrepresented, compared to the general population, among patients with specific conditions commonly occurring in people with CF. Some of the first positive reports noted congenital bilateral absence of the vas deferens (72). Other studies reported increased frequency of pancreatitis (73–75), allergic bronchopulmonary aspergillosis (76), aquagenic wrinkling of the palms (77), and bronchiectasis (78). Several reports, but not all, reported that CF carriers were at increased risk for asthma (79). Wang et al. found that among patients with rhinosinusitis, CF carriers were overrepresented compared to the rate that would be expected in the population (80). Similarly, Malagutti et al. found that patients with radiologic evidence of chronic sinusitis were more likely to be CF carriers than expected, compared to the general population (81). Despite being relatively small, these studies demonstrated that CF carriers are at increased risk for some CF-related conditions. One criticism of this approach is that some of the CF carriers under study could actually have a second undetected *CFTR* mutation. However, as more advanced screening approaches detect more *CFTR* mutations, this possibility is less likely. Another limitation of this study design is that it is not possible to simultaneously estimate the risk of the CF carrier state for multiple CF-associated diseases.

To address the limitations described above, population-based studies have been conducted. Two reports used administrative claims data to identify CF carriers, controls, and health outcomes. In a study of insurance claims from Iowa and South Dakota, 769 obligate CF carriers and CF carriers identified via genetic screening were more likely to experience a respiratory infection (pneumonia, sinusitis, etc.; $p = 0.028$) and experienced higher numbers of respiratory infections ($p = 0.039$). CF carriers were more likely to be prescribed an antibiotic used to treat respiratory infections ($p = 0.018$) and to have more of these prescriptions ($p = 0.035$) than controls (82). In a second, larger study, which included 19,802 CF carriers and 79,208 controls matched by age, sex, and enrollment period, Miller et al. found that with all respiratory infections considered, incidence rates were greater among CF carriers than controls (4). Examples include nontuberculous mycobacterial, aspergillosis-associated, and *Pseudomonas* infections. In fact, 57 of 59 CF-associated conditions considered (both infectious and noninfectious conditions) were significantly more common among CF carriers than controls. Examples of noninfectious diseases included asthma, bronchiectasis, type I diabetes/secondary diabetes, chronic pancreatitis, gastroesophageal reflux disease, male infertility, short stature/lack of normal expected development, cholelithiasis, and constipation; see **Figure 1** for a summary of these conditions (4). In addition, Çolak et al. considered only CF carriers with F508del mutations and found that CF carriers were at increased risk for bronchitis and bronchiectasis; bronchitis was defined by a survey, and bronchiectasis was based on inpatient diagnoses (83). While this study did not find the same increased risk for some conditions that others detected, the authors did not study infections in children, did not measure outpatient visits, and restricted their study population in a way that may have limited their ability to identify increased risk. Specifically, for each of the health conditions considered, patients were excluded if they presented that particular condition at the baseline examination, which would bias the result toward the null (83).

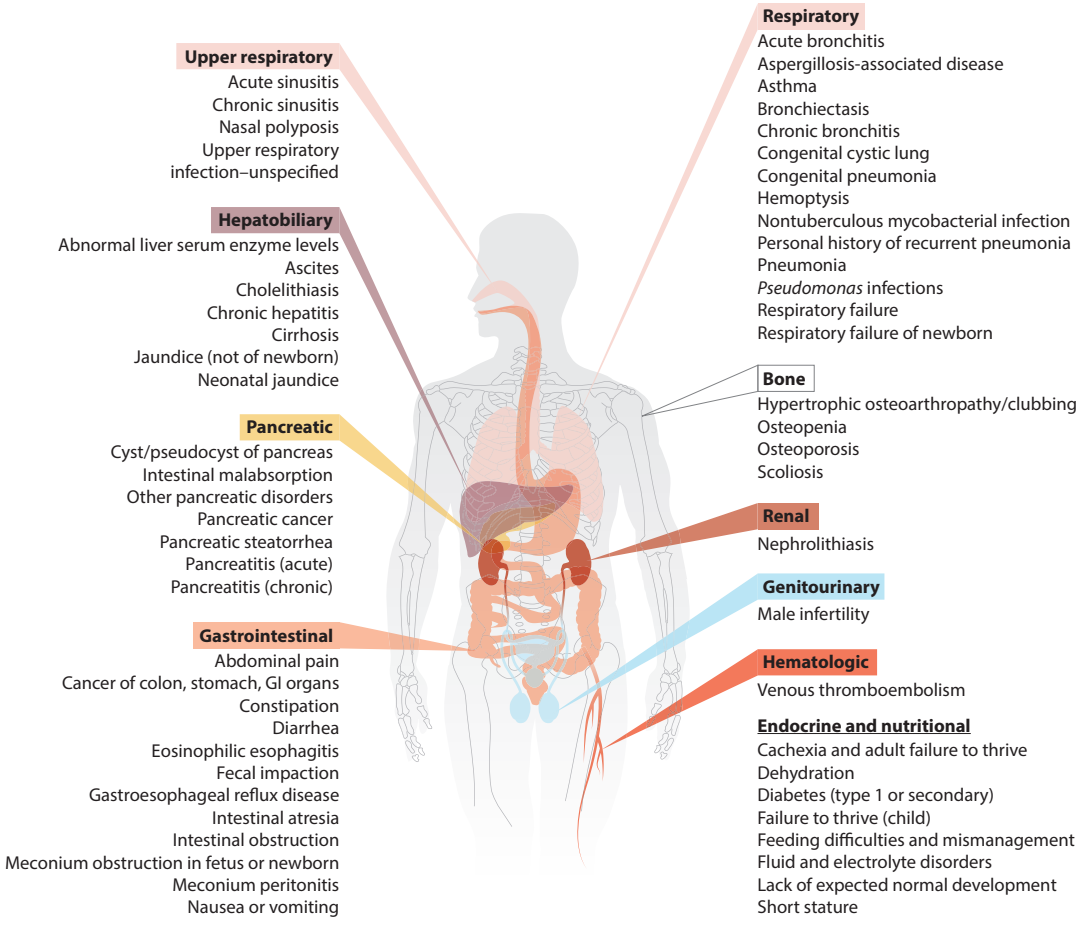

Figure 1

CF-related conditions for which CF carriers may be at increased risk. The figure summarizes results from Reference 4. Abbreviations: CF, cystic fibrosis; GI, gastrointestinal.

In recent decades, survival among people with CF has increased dramatically, and as survival has increased, so has the number of other conditions associated with CF. For example, an increased risk for colon cancer has been described (84, 85). CF carriers also appear to be at higher risk for multiple gastrointestinal cancers (84). In addition, a group that linked *CFTR* genotyping data to clinical records found CF carriers to be at risk for other cancers (86). Finally, CF carriers may be at risk for conditions that are not commonly reported among people with CF. Historically, the lifespan of people with CF has been limited, with very few people with CF surviving into their sixth decade; thus, people with CF may not live long enough to develop some diseases more common among older people. One example is cardiovascular disease. People with CF do have some risk factors for coronary artery disease. For example, CF is associated with endothelial dysfunction, a known independent risk factor and early indicator for coronary artery disease (87–89). Accordingly, CF carriers may also be at increased risk for cardiovascular disease.

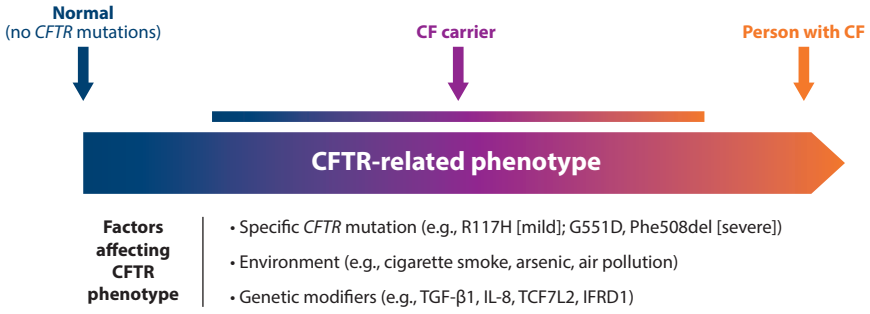

Figure 2

Factors potentially modifying cystic fibrosis (CF) carrier phenotypes.

CONCLUSIONS

The widespread availability of genetic testing for *CFTR* mutations has enabled larger population-based studies of heterozygotes. Collectively, these studies demonstrate that CF carriers are at increased risk for some CF-related conditions. It is increasingly likely that prior publications that claimed that CF carriers were not at increased risk may have been underpowered to detect such differences. However, the individual risk attributable to the CF carrier state has yet to be determined in prospective studies. In addition, the breadth of diseases associated with the CF carrier state is unknown. *CFTR* is widely distributed across multiple organ systems, so multiple diseases and syndromes need to be explored in detail. Also, multiple different factors could affect the CF carrier at an individual level (see **Figure 2**).

Considering the work highlighting CF carriers to date, several points are clear:

1. CF carriers have measurable differences in several biological variables [e.g., higher sweat chloride (51) and trypsinogen levels (63) and altered neutrophil function (59)]. In general, the levels of these variables are situated between levels found in people with CF and people with two normal *CFTR* genes.
2. CF carriers are at increased risk for some conditions associated with CF, such as chronic pancreatitis, diabetes, and bronchiectasis (4).
3. The risk of conditions that CF carriers experience is considerably lower than that experienced by people with CF (4), and many CF carriers may never develop any of the serious conditions commonly experienced by people with CF.
4. Both universal newborn screening and prenatal screening practices will increasingly identify more CF carriers. Accordingly, knowledge of *CFTR* genotype status will provide CF carriers an additional rationale to avoid specific health-related behaviors. For example, smoking can independently decrease CFTR activity (19, 20). Thus, CF carriers may be at higher risk for adverse pulmonary effects from smoking.
5. Knowledge of *CFTR* genotypes may help explain why some people contract unusual pulmonary infections (e.g., nontuberculous mycobacterial infections).
6. The CF carrier state may inform screening for some health conditions. People with CF are encouraged to undergo testing for some conditions at an earlier age (e.g., colon cancer); future investigations may support similar adjustments for CF carriers.

FUTURE DIRECTIONS

Future work should focus on developing more precise estimates of health risks attributable to the CF carrier state across the lifespan. CF carriers may require some additional genetic or environmental modifier to develop a particular disease. Studies may need to be large to identify other disease-modifying risk factors. In addition, such studies should not be restricted to health conditions associated with CF, nor should they solely focus on increased risks for particular diseases. Indeed, many have speculated that CF carriers may be less likely to acquire some diseases, given the high frequency of F508del.

Beyond epidemiological investigations, mechanistic studies designed to describe how decreased CFTR function may increase the risk forecast for different disease states are needed. This work will be especially important given the emergence of pharmaceutical therapies designed to improve CFTR function (90–95). Given that these CFTR-targeting therapies are effective for people with CF, they may also be of benefit for some CF-related conditions among CF carriers. Currently, use of such drugs to treat CF carriers is prohibitively expensive. Nevertheless, more data will help inform the feasibility of using CFTR modulators to treat severe and complex respiratory infections in CF carriers, such as some nontuberculous mycobacterial infections, which are resistant to multiple antibiotics and are currently very difficult and expensive to treat (96–98). Unlike CF patients, CF carriers may need such treatments for only relatively short periods of time to augment antimicrobial treatments. Alternatively, CF carriers with specific diseases may benefit from therapeutic approaches designed to increase the functioning of the roughly 50% of existing wildtype CFTR channels (99). This approach may provide a more feasible therapeutic opportunity not available to people with CF, for whom both *CFTR* alleles have deleterious mutations. Such future work is important because there are almost 10 million CF carriers in the United States alone. Because there are so many more CF carriers than people with CF, population-based attributable risks for CF carriers may actually be greater than for people with CF (100).

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The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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