Cystic Fibrosis Syndrome: A New Paradigm for Inherited Disorders and Implications for Molecular Diagnostics

In this issue, Castaldo et al. (1) describe the use of denaturing gradient gel electrophoresis to scan the CFTR gene in cystic fibrosis (CF) patients from Southern Italy who were negative for the common CF mutations. As expected, rare mutations were found, five of which were useful in population screening. These findings are particularly important for laboratories in Italy and in areas with families of Italian descent.

Advanced molecular techniques provide a double-edged sword because they often detect sequence changes whose deleterious natures are by no means established. In the CFTR gene, such variants may cause classic CF, elicit atypical phenotypes with similarities to CF, or have no consequence to health.

Among recent letters in the New England Journal of Medicine on mild phenotypic variants of CF (2–4), one suggested that “the diagnosis of cystic fibrosis is too nebulous to preserve in clinical practice and that perhaps, as is the case with Cushing’s disease and syndrome, we need to have both a cystic fibrosis ‘disease’ . . . and a cystic fibrosis ‘syndrome’ (which would include the pancreatic manifestations, congenital bilateral absence of the vas deferens, or lesser pulmonary manifestations)” (3). As improved mutation detection technologies enter the clinical laboratory and identify rare CFTR mutations, labora-
torians and clinicians must be cognizant of the changing nature of genotype-phenotype associations.

The severity of the pancreatic and hepatic features of CF is highly variable. The gastrointestinal presentations endured by CF patients take many forms (5). The majority of patients (85–90%) experience pancreatic exocrine insufficiency. Those who retain pancreatic exocrine function (10–15%) typically carry at least one of the CFTR gene mutations that have been associated with residual function. Other CF patients have impaired endocrine insulin production associated with diabetes (10–15% of patients) or liver dysfunction (5), although no specific CFTR gene mutations have been associated with hepatic or endocrine pancreatic dysfunction.

Other hallmarks of CF, such as pulmonary disease and sweat electrolytes, also vary among patients. Frequencies of Pseudomonas aeruginosa and/or Staphylococcus aureus infections differ substantially, as do the rates of decline of pulmonary function. Particular mutations appear to be determinants of milder lung disease (6, 7). Even the benchmark diagnostic marker of CF, increased sweat electrolytes, is variable. Although most patients with classic CF have sweat chloride values >60 mmol/L, a minority of patients, often with atypically mild disease, have sweat chlorides in the reference range of ≤40 mmol/L (5, 6).

CFTR gene mutations have been implicated in the molecular etiology of other disorders with clinical similarities to CF. Congenital bilateral absence of the vas deferens (CBAVD) is the physiological basis of infertility in males with CF. Among non-CF men, CBAVD is thought to account for ~2% of all male infertility (8). Many men who exhibit CBAVD without lung or gastroin-testinal manifestations of CF have CFTR gene mutations (9–11). An important molecular finding in these men was the increased incidence of an intronic splice variant. The 5T allele of the variable-length polyypyrimidine tract preceding exon 9 is a suboptimal recognition element for the spliceosome and leads to the predominant loss of exon 9. A nonfunctional protein results from translation of the aberrant transcript and CBAVD may arise. The 5T allele appears to be 4 to 6 times more common among CBAVD CFTR alleles than among controls (10, 11).

The realization that CFTR gene mutations are a prominent molecular basis of isolated CBAVD led to the discovery that many of these men exhibit subtle, CF-like, sinopulmonary abnormalities not reported in their initial urological review. In conjunction with molecular analyses, Osborne et al. (12) noted defective chloride conductances across the nasal epithelial membranes, a hallmark of CF, in men with isolated CBAVD. Casals et al. (13) reported that 19 of 40 CBAVD men exhibited some symptoms of non-CF respiratory disease, e.g., rhinitis, sinusitis, or nasal polyps. With sinopulmonary abnormalities now apparent in a portion of CBAVD men, the genotype-phenotype questions continue. Might these mild, chronic respiratory complaints themselves represent alternative expressions of disease associated with CFTR mutations? CFTR mutations and/or the 5T allele have been associated with disseminated bronchiectasis (14), and perhaps with isolated nasal polyposis or sinusitis (15, 16).

Our laboratory has analyzed individuals with a variety of ill-defined sinopulmonary complaints (17). None of the 86 individuals studied had two CFTR mutations identified after screening for 30 common and/or mild alleles. Thirty-five of the 172 alleles (~20%) carried a CFTR mutation (chiefly ΔF508), compared with an expected 2% in the general population. Most interestingly, the frequency of the 5T allele was 12% in this group of patients, compared with 5% in the general population (P < 0.01), suggesting that 5T might play a role in atypical sinopulmonary disease analogous to that reported for isolated CBAVD. Similar data have been presented by Kerem et al. (18). In toto, the definition of CF from a pulmonary vantage point may encompass a broader range of disease, with concomitant impact on the delivery of clinical and molecular diagnostic services.

The expanding definition of the CF syndrome includes...
idiopathic chronic pancreatitis (ICP). A minority of CF patients who are pancreatic sufficient endure episodes of severe pancreatitis (5). Although multiple explanations for ICP can be postulated (heavy alcohol use, pancreatic cancer, autosomal dominant hereditary pancreatitis, hyperlipidemia, and others), CFTR dysfunction was investigated as a potential cause of ICP by two independent groups. Cohn et al. (19) examined 27 adults with ICP for whom postulated risk factors had been excluded. Ten patients were found to have at least one CFTR gene mutation or the 5T allele, and three of these individuals had two mutations. This high frequency strongly suggests an association between CFTR abnormalities and ICP. Similar data were reported by Sharer et al. (20).

The appearance of genotypes previously described in milder CF and CBAVD among the ICP patients raised the possibility that other aspects of the greater CF phenotype might have gone unnoticed in these individuals. In the paper by Cohn et al. (19), a male patient with an aberrant genotype (AF508/R117H,7T,9T) had CBAVD and smooth *P. aeruginosa* in his sputum. One of the AF508/5T women had borderline sweat electrolytes and impaired pulmonary function, whereas the other, with no overt pulmonary manifestations, had an older sister diagnosed with mild CF.

Impaired CFTR function has been implicated in unilateral absence of the vas deferens, obstructive azoospermia, and severe oligozoospermia in men (21–23) as well as hypofertility in CF women (24). CF and/or CFTR mutations have also been associated with isolated hypotonic dehydration (25) and increased sweat chlorides without any CF symptoms (26). With these observations, clinicians should consider a new definition of a CF syndrome that accommodates not only the breadth of respiratory symptoms now believed to arise from CFTR dysfunction, but the apparent heterogeneity of gastrointestinal disease, urogenital abnormalities, and other complications as well.

A confounding effect of improved laboratory diagnostics, including complete gene sequencing, is the detection of rare and conservative substitutions for which a consequence to health is not immediately apparent. In contrast to nonsense and frameshift mutations, missense alleles, distant intronic variants, and promoter mutations are less readily identifiable as the agents of disease. If a particular missense allele is relatively common, an association with a disease state might be statistically attainable. When such an allele is rare, however, elaborate in vitro functional studies are required. This has been performed on a few rare CFTR mutations and polymorphisms (27, 28), but is too cumbersome to fully address the allelic heterogeneity seen in CF.

Deep intronic sequence variants and promoter mutations represent their own challenges. The former represent a minority class of splicing mutations. Although documented in CFTR (6) and other genes, there is likely a negative ascertainment bias because few laboratories investigate introns far from coding regions. No clinical consequence can be attributed to mutations that remain undetected. The possibility of promoter mutations in CFTR has been raised but not yet substantiated.

Certainly, the 5T allele is a population risk factor for various aspects of the CF syndrome, but its incomplete penetrance limits its prognostic or diagnostic value for individual patients. Thus, testing for 5T is only appropriate in specific situations, such as for men with a prior diagnosis of CBAVD. Clinical testing for 5T in patients with other disease manifestations for which its role is only theoretical is discouraged.

The interpretation and use of CFTR testing will ultimately involve not only CFTR mutations, but epigenetic factors as well. The impact and/or penetrance of a particular CFTR allele may be influenced by environmental factors, as well as by other genes and the variants they harbor. Indeed, the concept of a CF modifier gene is not new (29). These external influences might impact not only individuals with two genuine or putative CFTR mutations, but atypical disease patients with only one mutation as well.

In summary, the advances in genotyping technologies have changed our appreciation of phenotypic heterogeneity, as evidenced by the developments described in CF. We are now in the position of allocating valuable resources to those patients who are most likely to benefit from these efforts. For example, do we completely sequence CFTR in mutation carriers who possess some phenotypic abnormality but clearly do not have CF? What if a new missense mutation is found? How much effort should be devoted to studying these unknown variants/mutations? As the human genome project nears completion in 2003, what other genetic disorders will follow a similar paradigm? The forces that lead to the disparate manifestations of the CF syndrome are likely to be multifactorial in nature. The challenge for the future will be the identification of all modifying factors and their distillation into a formula that will predict disease outcome with accuracy. Although this is a daunting task at first glance, it pales in comparison to accomplishments of the last 20 years.

References


