

Commentary

Modifier genes and variation in cystic fibrosis

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Abstract

The availability of molecular tools to carry out genotyping has led to a flurry of association studies between specific genes and clinical indices of disease or disease susceptibility. Human studies, for the most part, have a limited number of subjects available, precluding whole genome types of approaches. 'Candidate gene' strategies have consequently become widespread, probably in part due to the inherent similarity to clinical association studies. Such studies in cystic fibrosis have found tantalizing results in genes involved in infection and inflammation, but many other relevant pathways remain untapped. Genome scanning approaches may eventually uncover genes not currently recognized as important to cystic fibrosis. In the meantime, while thousands of polymorphisms are cataloged and other genomic resources become more available, the number of association studies with candidate genes will no doubt increase. To make sense of these studies, the choice of gene and phenotype must be carefully considered.

Keywords: genetic variation, infection, inflammation, ion transport, pulmonary function

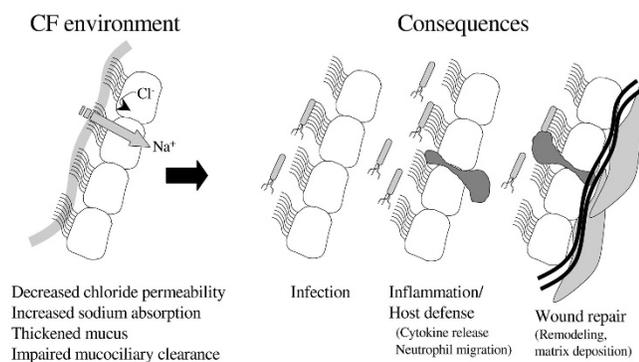
Introduction

Rozmahel *et al* [1] reported, in 1996, that the lethality associated with a knockout allele of the murine cystic fibrosis transmembrane conductance regulator (CFTR) could be modified by different strain backgrounds. They genetically mapped a locus conferring the modifying effect to chromosome 7. Because the predominant manifestation of disrupting CFTR in the mouse is intestinal obstruction, a feature of cystic fibrosis (CF) in humans, a region of the human genome on chromosome 19 corresponding to the mouse locus was tracked in siblings with CF. Zielenski *et al* [2] thus found the occurrence or absence of meconium ileus, a perinatal form of intestinal obstruction, coincided with haplotypes of chromosome 19 inherited by the siblings. This coincidence implied that the gene conferring resistance or susceptibility to intestinal obstruction in mice is also polymorphic in humans,

and that those polymorphisms have a similar effect on CF patients to that which strain variants of these genes have on CF mice.

Whereas the identity of the gene, or genes, responsible for this phenomenon has not yet been determined, this study illustrates several important points. Firstly, it demonstrates that some of the clinical variation between CF patients is genetic, but conferred by genes other than that for CFTR. It importantly also indicates that relatively common genetic variation, with little or no overt phenotypic effect on the general population, can have a significant effect in the context of CF.

This is an important precedent for CF, but the effect of this particular locus seems restricted to the gut. CF, however, involves multiple organs whose common feature

Figure 1

Potential sites for the effects of CF airway modifiers. Genes that affect the expression of CFTR, processing of the CFTR protein, or activity of ion channels in the epithelium, as well as control of mucus production and mucociliary clearance (left), could all have an impact on the sequence of events in the CF lung. Genes that influence the consequences of the defects at the epithelium, such as infection, chemotactic signals of inflammation, and tissue remodeling, would also be likely to modify clinical manifestations of the disease.

is epithelium expressing CFTR, and most morbidity and mortality is due to the complications in the respiratory tract. Decreased mucociliary clearance, viscous secretions and glandular plugging by these secretions, and altered ion transport are all thought to contribute to the milieu that allows colonization by opportunistic species of bacteria, such as *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Burkholderia cepacia*. Associated with the chronic infection is an overwhelming inflammatory response that, together, lead to destruction of the airways. Clinical and basic research regarding CF is consequently focusing on the airways, but identifying effective sites for intervention remains elusive (see [3]).

Searching for modifier genes of pulmonary disease is one approach to identify therapeutic targets. The identification of genes that impact on the severity of CF airway disease also illuminates therapeutic targets, either the protein product of the gene or the entire pathway in which it acts.

Phenotype: a critical consideration

A genetic approach can certainly be powerful, but not without caveats. As in any genetic study, the keystone is the phenotype. Without a robust, reliably measured or scored phenotype, a genetic effect is difficult to resolve. Therein lies the biggest hurdle for CF: What is a relevant, variable phenotype that can be accurately and reliably measured or scored? As in the meconium ileus example, one could classify a trait into categories, such as mild or severe, and determine whether associations exist between alleles of a particular gene and a phenotypic category. Unfortunately, pulmonary disease in CF appears as a continuum of severities, unlike the present or absent pheno-

type of meconium ileus. There is also a temporal component to the disease that confounds scoring [4]. Colonization of the lungs by bacteria, for instance, is a consistent feature of CF. Virtually all patients become colonized at some point, although some acquire infection early in life and some quite late. Therefore, if one wishes to use microbiology as a phenotype influenced by a modifier gene, scoring simply by whether a patient is colonized is likely to be inadequate. It would rather be more differentiating to use criteria such as age at which a patient becomes colonized, bacterial species, and so on. Pulmonary function similarly declines in all patients, but the profile of decline varies from patient to patient [4,5], with the greatest differentiation obvious at older ages. This creates an inherent difficulty in using pulmonary severity as a phenotype. Patients are of all ages but, at the ages for which the greatest resolution of severity is possible (ie late teens and older), many of the severely affected patients are no longer alive. Many studies comparing pulmonary severity are therefore limited to patients' latest pulmonary function measurements.

The most relevant clinical phenotype is survival, but the number of potential contributing factors (genetic and non-genetic) is great and may dilute each other in an analysis. It is therefore desirable to look at more specific traits that convincingly contribute to the pathophysiology of the disease. From the basic defect, genes that act on CFTR gene expression, protein processing, protein turnover or CFTR activity could all impact on disease. In the airway, genes modifying the downstream effects of CFTR mutations, such as infection susceptibility, impaired mucociliary clearance, enhanced inflammatory response, and remodeling of the airways (fibrosis), could all influence the disease course (see Fig. 1). There are other conceptual considerations as well. A CF modifier, for instance, may only modify in a CF context, as appears to be the case for the meconium ileus modifier. The modifier may alternatively modify a trait in healthy and CF-affected individuals, but the clinical significance of the effect is greater or exclusive to CF.

Candidate gene examples

The presented considerations bear on the choice of approach. Garred *et al* [6], for example, reasoned that genes involved in innate defense against bacteria outside CF might also be relevant in the context of CF. They examined CF patients for alleles of the mannose-binding lectin (MBL) gene associated with reduced serum levels of MBL. MBL is a member of the collectin family of proteins, participating in innate defense against bacteria [6]. This and a similar study [7] showed that pulmonary function was significantly lower in patients carrying low-expression MBL alleles. Survival predictions based on pulmonary function profiles indicate that patients with the low-expression MBL alleles will have a shorter lifespan than those with high-expression alleles [6].

Genes of the major histocompatibility complex play a role in immune defense and allergic responses. Aron *et al* [8] consequently looked for associations between class II human leukocyte antigens (HLA) and indices of pulmonary disease in CF. The HLA gene alleles showed no association with most traits, but presence of the DR7 allele did show some correlation with *P aeruginosa* colonization.

Another player in the host defense system is nitric oxide (NO). Expired NO levels are reduced in CF. NO may have some antimicrobial role against *P aeruginosa* so this decrease may have serious functional consequences [9,10]. Expression of the NOS2 gene is downregulated in CF airway epithelial cells [9,10], perhaps accounting for the reduced NO levels in CF. However, another nitric oxide synthase gene, NOS1, shows significant airway expression and its alleles were evaluated for association with pulmonary disease. Grasmann *et al* [11] found that an intronic trinucleotide repeat in the NOS1 gene not only associated with differences in exhaled NO (greater repeat number is associated with lower NO levels), but that colonization by *P aeruginosa* and *Aspergillus fumigatus* also associated with the high repeat number.

Associated with infection is inflammation, a process thought to contribute significantly to the destruction of the lung (reviewed in [3]). Polymorphisms in genes involved in infection and inflammation are numerous (for reviews, see [12–14]), which prompted Hull and Thomson [15] to look at several genes that are probably important in CF airway inflammation, namely those encoding the cytokine tumor necrosis factor- α (TNF- α) and the anti-oxidant glutathione-S-transferase-M1 (GST-M1). While no association was found for GST-M1 alleles, a high-expression TNF- α allele was found to be associated with lower body mass index and pulmonary function. The gene for transforming growth factor- β 1 (TGF- β 1), a cytokine with many effects (pro-fibrotic [16], anti-inflammatory [17]), was similarly studied with regard to its effect on CF pulmonary function. Patients carrying an allele associated with high expression of TGF- β 1 had a more rapid decline in pulmonary function than patients carrying low-expression alleles, from which the authors implied that the profibrotic function of TGF- β 1 dominates its effect on the CF lung [18].

The CF airway modifiers examined thus far have consisted of candidate genes chosen because of their suspected role in CF pathophysiology and because they have identified polymorphisms. The gastrointestinal manifestations of CF, however, show that other approaches should also be pursued. In addition to the meconium ileus modifier identified by traditional genetics, pathways that influence mucin expression are predicted to be CF gut modifiers based on a transgenic mouse model. Parmley and Gendler [19] found that CF mice on a background in which the *Muc1* gene is knocked out did not experience the intestinal

obstruction characteristic of CF animals and so, while not identified by polymorphisms, the *Muc1* gene clearly modifies the CF phenotype.

Conclusion

The literature is full of modifier-type studies for many diseases, but this is just the beginning. The genome sequencing efforts are identifying thousands of polymorphisms (see [20]), thereby providing the potential to study, in the near future, virtually any gene of suspected interest. These studies will provide associations but, because of the number of comparisons that will be possible, chance associations are a certainty. Results must therefore be interpreted with caution. It must be remembered that a candidate gene approach is one of association, not cause and effect. The opportunities are nonetheless exciting and hold much promise toward our understanding of CF pathophysiology and the potential to thwart it.

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