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Update on gene modifiers in cystic fibrosis

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Abstract

Purpose of review—Cystic fibrosis (CF) is a common, life-limiting monogenic disease, which typically manifests as progressive bronchiectasis, exocrine pancreatic dysfunction, and recurrent sinopulmonary infections. Although the gene responsible for CF (*CFTR*) was described in 1989, it has become increasingly evident that modifier genes and environmental factors play substantial roles in determining the severity of disease, particularly lung disease. Identifying these factors is crucial in devising therapies and other interventions to decrease the morbidity and mortality associated with this disorder.

Recent findings—Although many genes have been proposed as potential modifiers of CF, only a handful have withstood the test of replication. Several of the replicated findings reveal that genes affecting inflammation and infection response play a key role in modifying CF lung disease severity. Interactions between *CFTR* genotype, modifier genes, and environmental factors have been documented to influence lung function measures and infection status in CF patients.

Summary—Several genes have been demonstrated to affect disease severity in CF. Furthermore, it is likely that gene–gene and gene–environment interactions can explain a substantial portion of the variation of lung disease. Ongoing genome-wide studies are likely to identify novel genetic modifiers. Continued exploration of the role of genetic and nongenetic modifiers of CF is likely to yield new options for combating this debilitating disease.

Keywords

cystic fibrosis; genome-wide; interaction; modifier gene

Introduction

Cystic fibrosis (CF) is an autosomal recessive disorder affecting over 70 000 individuals worldwide and leads to significant morbidity and mortality with a median predicted age of survival of 37 years in 2006 (www.cff.org). The gene responsible for CF, *CFTR*, was identified in 1989 [1,2]. Phenotypic variation in a monogenic disorder, such as CF, can be attributed to several sources, including different mutations in the disease-causing gene, genetic modifiers, environmental factors, random events, and interactions among and between any of these sources (Fig. 1Fig. 1). For some manifestations of CF, such as exocrine pancreatic insufficiency, *CFTR* genotype correlates with variation in organ function. However for other manifestations of CF, phenotypic variation remains substantial, even among patients with

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identical mutations in *CFTR*. The latter is demonstrated by the wide variation in lung function observed in patients carrying the same *CFTR* mutations (ΔF508 homozygotes) [3–5]. Identifying sources of disease variation is crucial to improving care for individuals with CF. This knowledge may improve the efficacy of current symptomatic therapies as well as identifying patients at risk for poor outcomes. New therapeutic targets may be discovered by this approach and some of these targets may be more accessible than *CFTR*. An additional impetus for elucidating the modifiers of a monogenic disease, such as CF, is the potential insight into factors that underlie variation in lung function in polygenic disorders, such as asthma. Often the first step in distilling the sources of disease variation is determining the relative contribution of genes (i.e., heritability) versus environmental factors. Classically, twin and sibling studies have been widely used to assess the degree of heritability of various phenotypes. In CF, twin studies have demonstrated that there is a genetic contribution to lung disease (heritability estimates: 0.54−1.0) [6,7•], diabetes (~1.0) [8], and meconium ileus (~1.0) [9], but not distal intestinal obstruction syndrome [9]. Not only can heritability studies justify the search for genetic modifiers, particularly for lung disease, they also provide a quantitative estimate of the effect of environmental modifiers and gene–environment interactions.

Candidate gene studies

Having identified manifestations of CF that are more prone to be influenced by genetic variation, such as lung function, the next step is to identify modifier genes. Candidates are generally selected on a mechanistic basis either because they function in key disease-related pathways or have been previously identified as modifiers in similar diseases. For example, *TGFB1* has been implicated as a modifier of respiratory disease [10–16], and *PPARγ* as a modifier of diabetes risk [17]. Linkage studies can help to identify regions of interest within the genome associated with particular CF phenotypes in order to target searches for candidate genes [9,18] (L.L. Vanscoy, unpublished observation). Genetic modifiers have also been evaluated by association analysis using case–control or family-based study designs.

A number of candidate genes have been tested for association with different CF phenotypic manifestations. Published studies have examined mostly immune or inflammatory genes, including *ACE* [19,20], *ADRβ2* [20–23], *ATB*^{0} [24], *CAPN10* [25], *ClCN2* [26], *DEFβ4* [27], *ENaC* [28], *FcβRII* [29], *GCLC* [30], *GSTM1* [20,31–35], *GSTM3* [34], *GSTP1* [20,33–35], *GSTT1* [34], *HFE* [36,37], *HLA-I* [38], *HLA-II* [38–41], *HLA-III* [38], *HSP70* [39], *IFN-γ* [19], *IL1-β* [39], *IL6* [25], *IL10* [19,20,42], *IL18* [25], *KCNJ11* [25], *MBL2* [20,43–52,53••], *MIF* [54], *NOS1* [55–57], *NOS3* [20,58], *MASP-2* [48,51], *PPARβ* [25], *SERPINA1* [20,59–67], *SERPINA3* [68], *SFTPA1* [50], *SFTPA2* [50], *TLR4* [69], *TGFB1* [19,20,52,53$^{\beta\beta}$,67,70–72••,73••], *TNFα* [19,20,25,32,52,74–76], *TNFα*-receptor [28], and *TNFβ* [39].

One of the advantages to candidate gene association studies is their less costly and less resource intensive means of obtaining data than genome-wide approaches. Additionally, studying candidates within known mechanistic pathways increases the likelihood that off-the-shelf therapies already exist to alter the pathway if an association is found. However, the candidate gene approach, being restricted to known pathways, does not allow for the discovery of novel pathways that affect disease. Furthermore, there exists a bias toward false-positive results as any positive association (even in underpowered studies) may be considered relevant as the candidate gene was selected from a pathway suspected of being linked to disease. Given the relatively low number of patients in most studies (<500) and the likely low magnitude of effect size for many modifier genes, replication of results in independent populations is essential. Of the aforementioned studies, only 13 candidate genes have been studied in two or more separate populations of individuals with CF. This review focuses on seven candidate genes in which at

least three separate studies have examined the effect of a polymorphism on a particular phenotype (Table 1 Table 1); the majority of studies examine the phenotype of lung function.

Transforming growth factor β1

TGFB1, a cytokine and mediator of fibrosis, has been studied in a number of lung diseases besides CF. Polymorphisms in *TGFB1* regulate levels of transforming growth factor β1 (TGFβ1) production and have been shown to modify the development and/or severity of lung disease in fibrotic lung disease [77,78], asthma [10,13,15], and chronic obstructive pulmonary disease (COPD) [11,12,14]. At least three polymorphisms have been investigated for their role in CF, including one in the promoter region (−509C>T) and two in exon 1, Leu10Pro (c869C>T) and Arg25Pro (c915G>C). No associations have been observed between codon 25 variants and lung disease in several studies [19,20,70,71]. Polymorphisms at the −509 promoter site and codon 10 may affect lung function; however, neither variant has been observed to affect infection status. Studies by Drumm *et al.* [20] and Bremer *et al.* [73••] have demonstrated that the −509T allele is associated with improved lung function, whereas Brazova *et al.* [71] did not observe any association. Differing results with reference to lung phenotypes have been found with codon 10; better-powered studies suggest that the T allele correlated with higher lung function [20,73••]; however, other studies have reported lower function with the T/T genotype [70], a steeper decline with both the T/T and C/C genotypes [53••], and two studies reported no association between genotype and lung function [19,71]. Together, these studies suggest that *TGFβ1* is a modifier of CF lung function, and the best-powered studies are consistent for association of the −509C allele and the codon 10 T allele with improved lung function.

Other manifestations that correlate with the severity of CF lung disease (e.g., colonization or the age of acquisition of *Pseudomonas* or *Burkholderia cepacia*) do not show associations with any of the three *TGFB1* polymorphisms [19,53••,70,71]. More limited literature suggests that none of the polymorphisms affect survival [19,52,70]. Replication in some studies [73••], but not others, could be due to the differences of these studies to detect low effect variants. Additionally, other variables such as age or cohort effects may confound associations [53••, 79•]. Finally, differing results could also be due to the presence of unaccounted for candidate gene interactions with *CFTR*, other genetic modifiers, or environmental factors. For *TGFB1* codon 10 variants, interactions have been documented with *MBL2* [53••], and for both the −509 promoter site and codon 10 interactions have been reported with *CFTR* genotype (ΔF508 homozygote versus not) [73••], and the environmental factor of secondhand smoke exposure in the home [72••]. In summary, polymorphisms of *TGFB1* modify CF lung function and interactions with other genetic and environmental factors modulate the modifier effect of this gene.

Mannose binding lectin 2

Mannose binding lectin 2 (*MBL2*), which codes for the collectin MBL, plays an important role in innate immunity by promoting phagocytosis of infectious organisms [80]. Polymorphisms in *MBL2* are associated with differing levels of MBL production [81], and deficiencies in MBL production have been linked to an increased incidence of infection, including pneumonia and sepsis, even in patients with no defects in adaptive immunity [80]. The polymorphisms most frequently studied include three amino acid substitutions in exon 1 (52, 54, 57) and one in the promoter (−221G>C). By convention, an exon 1 haplotype is classified as ‘A’ if all three codon variants are wild-type and ‘O’ if any one of the three variants is not wild-type. O variants typically lead to decreased or deficient production. The −221 promoter variant (X/Y) is often analyzed in the context of the exon 1 haplotype, with genetic combinations of the promoter and exon 1 variants frequently being categorized into three groups: low or deficient producers,

intermediate producers, and high producers. Only one study [48] has been published on the role of the −550G>C promoter variant (H/L) in CF.

MBL-deficient states in CF patients have been associated with reduced survival, most likely secondary to more severe lung disease, but not necessarily due to increased susceptibility to infection. Two studies examining survival [44,52] found that it was decreased in patients with insufficient MBL states as defined by exon 1 genotypes. The relative deficiency of serum MBL has been shown to be associated with accelerated lung function decline in individuals with CF [82]. The majority of candidate gene studies [43,44,46,47,49,53••] have documented worse lung disease with insufficient *MBL2* genotypes; however, a few studies [20,48,50] have seen no effect, and one study [51] saw worse lung function with high or intermediate producers. Most studies [43,44,46,47,51] have seen no effect of *MBL2* variation on colonization or the age of acquisition of *Pseudomonas*; however, other studies [49,53••] have observed earlier acquisition of *Pseudomonas* with deficient states or conversely with sufficient states [48]. *MBL2* genotype was found not to affect *B. cepacia* colonization in two studies [46,51], but colonization was found to be more likely with A/O or O/O genotypes in another study [44]. In summary, genotypes linked with decreased MBL production appear associated with lower lung function that likely is the cause of the observed increased mortality.

Tumor necrosis factor α

Polymorphisms of tumor necrosis factor α (*TNFα*), an inflammatory cytokine, have been associated with the development of asthma [83] and COPD [84] in meta-analyses. These polymorphisms are associated with differing levels of TNFα production, and in individuals with CF, higher sputum TNFα levels have been associated with decreased lung function [85]. Several polymorphisms of *TNFα* have been investigated as potential modifiers of CF including three within the promoter region, −851C>T [74], −308G>A [19,20,32,52,74–76,86], and −238G>A [52,74], and 1 in intron 1, +691G ins/del [74]. However, only −308G>A has been studied multiple times. Published studies suggest that this polymorphism does not affect lung function, infection, diabetes, or survival, though results are mixed for nutritional status. Most studies [19,20,74,76] have seen no effect of the −308G>A polymorphism on lung disease, although one study [32] did report worse function with the G/A genotype. Similarly, most studies [19,32,74,76] have not seen any genotype effect on the acquisition or colonization of *Pseudomonas*, although one study [75] has reported that earlier acquisition and colonization with *Pseudomonas* is more likely with the G allele. More limited literature suggests that the −308G>A polymorphism does not affect survival [19,52] or the prevalence of diabetes [19, 76,86]. Although two studies [74,76] have reported no effect of the polymorphism on weight or BMI, another study [32] reported that the G/A genotype was associated with a decreased weight *z*-score. In summary, the *TNFα* −308G>A polymorphism does not appear to modify some of the more common manifestations of CF; however, the role of other variants of *TNFα* is uncertain due to lack of replication.

α_1-Antitrypsin

Over 100 mutations have been identified in α_1-antitrypsin (*SERPINA1*) [87], a gene coding for the proteinase inhibitor α_1-antitrypsin. Three commonly studied mutations include the S mutation in exon 3 (Glu264Val or c2313T>A) and the Z mutation in exon 5 (Glu342Lys or c4627G>A), which result reduced plasma levels of α_1-antitrypsin, and +1237G>A in the 3′-untranslated region, which may result in a decrease in acute phase levels [60]. Deficiency of α_1-antitrypsin is associated with COPD. Available literature suggests that the Z, S, or +1237G>A polymorphisms do not affect lung disease or nutritional status and have mixed results regarding infection status and liver disease. Most studies [20,59,62,64,65] have seen no effect of these polymorphisms on lung function; however, one study [60] reported better

function with an S or Z mutation, and another study [66] reported worse function with the G/G genotype at position +1237.

Most studies [60,63,64] have reported no association between the age of acquisition of or colonization with *Pseudomonas* with genotypic variants of the +1237 position, although one study [62] reported colonization was more likely with the G/G genotype. Two studies [59, 64] have reported that earlier acquisition and higher prevalence of *Pseudomonas* are seen with the S and Z mutations, other studies [60,63] have not observed similar findings. Although two studies [60,62] did not see any effect on the prevalence of liver disease, a more recent study [67] found an increased prevalence of the Z mutation in CF patients with liver disease. More limited literature suggests that polymorphisms in *SERPINA1* do not affect nutritional status [64,66]. In summary, mutations in *SERPINA1* seem unlikely to affect the more common manifestations of CF, but may alter the susceptibility to the development of hepatobiliary complications.

Glutathione S-transferase M1 and glutathione S-transferase P1

The null mutation in glutathione S-transferase M1 (*GSTM1*), an antioxidant gene, results in no protein production and may lead to decreased lung function growth in children [88]. Polymorphisms in glutathione S-transferase P1 (*GSTP1*), another antioxidant gene in the glutathione-S-transferase family, have been associated with susceptibility to asthma [89–91]. The four studies that have examined the role of *GSTM1* (null deletion) and *GSTP1* (Ile105Val (c1375A>G) polymorphism) in CF suggest that the null deletion does not affect lung function, infection status, or nutritional status, and the polymorphism c1375A>G does not affect lung function. Three studies [20,32,34] demonstrated no effect on lung function for both genes; one study [35] reported an interaction between *GSTM1* wild type and the *GSTP1* Ile105Val genotypes, but it is unclear whether this genetic combination is overrepresented in a group of individuals with severe CF lung disease for protective or detrimental reasons. More limited literature suggests that the null deletion does not affect the prevalence of colonization with *Pseudomonas* or nutritional status as measured by weight or BMI *z*-scores [32,34].

β-Adrenergic receptor

β-Adrenergic receptor (*ADRβ2*) codes for an airway receptor that modulates reactivity; its pharmogenetic effects in asthma are still unclear [92]. The four studies that have examined the role of *ADRβ2* in CF have focused on the Arg16Gly and Gln27Glu polymorphisms; one study [21] has examined the Thr164Ile polymorphism. In regard to clinical phenotypes, only effects on lung disease were investigated by more than one study. Three studies [20,22,23] saw no association of lung disease with the codon 16 or codon 27 polymorphisms, although one study [21] reported worse function and decline in individuals who carried a Gly/Gly or Arg/Gly genotype at codon 16 or a Glu/Glu or Gln/Glu genotype at codon 27.

Interactions

No gene operates in a vacuum, and although several CF modifier genes have been identified by association and will continued to be identified through genome-wide studies, it is likely that most modifier genes in isolation confer small effects upon phenotypic variation, analogous to the effect of common variants in common diseases. However, genetic modifiers in concert with other factors such as *CFTR* genotype, variants in other modifier genes, or environmental exposures are likely to have more substantial and therefore detectable effects. Several interactions have been reported to date and two have been replicated in independent populations: an *MBL2–CFTR* interaction and an *MBL2–Pseudomonas* interaction.

Gene–gene interactions

To date several gene–gene interactions have been reported between *CFTR* and modifier genes, including *GCLC* [30], *MBL2* [49,50], *FCγRII* [29], *NOS1* [55], and *TGFβ1* [19,73••]. Most of these reported gene–*CFTR* interactions demonstrate the presence of or increased variant polymorphism effects in the context of ΔF508 or other severe mutation homozygosity, excepting the *GCLC–CFTR* interaction and the *TGFB1–CFTR* interaction as reported by Bremer *et al.* Other reported gene–gene interactions in CF include *MBL2–TGFB1* [53••], *TNF*•-8.1 ancestral MHC genotype [75], and *GSTM1–GSTP1* [35].

With the candidate gene approach, testing for gene–gene interactions is achievable as most modifier gene studies have typed only *CFTR* and a few other select polymorphisms. However, with the new technology to obtain genome-wide data, the number of genes in a study that uses a genome-wide approach will have increased exponentially. One current challenge in the field of modifier gene studies is developing analytic tools to determine which of potentially millions of permutations of gene–gene combinations result in biologically significant interactions.

Gene–environment interactions

Identifying gene–environment interactions in CF could prove to be clinically valuable as environmental factors may be more amenable to interventions than altering the functions of genetic modifiers. However, accurately describing and quantifying environmental exposures is more challenging and more time-consuming than genotyping. Furthermore, environmental exposures generally occur along a continuum, and often for the simplicity of analysis, these truly continuous variables are divided into discrete variables with the potential introduction of bias to simplification of a continuous variable. Also, the analytic tools for identifying gene–environment interactions using genome-wide data are still in their infancy.

It can be hypothesized that adverse effects seen with particular variant polymorphisms are amplified in the presence of adverse environmental stimuli. To date, only a handful of gene–environment interactions have been identified; these include interactions between *MBL2* and *Pseudomonas* [44,49], and *MBL2* and *Staphylococcus aureus* [48] leading to additional decreases in lung function. Other than infections, published data on interactions with known environmental modifiers of CF, for example, the effect of air pollution on CF lung function [93], is virtually nonexistent. However, recently interactions between secondhand smoke exposure in the home and *TGFB1*, as well as *CFTR*, have been identified [72••].

The few CF studies that have considered environmental exposures in studying interactions have focused on risk factors. In the future, it is expected that studies will consider treatment modalities as environmental exposures. Identification of genotypes that respond to particular interventions may lead to improvements in customizing therapies for individuals. Asthma research has already identified a number of genetic polymorphisms that modulate the response of individuals to specific treatment modalities, including β2-agonists, glucocorticosteroids, and leukotriene modifiers [94]. As a similar example in CF, VX-770, which is in phase II trials as of early 2008, is being tested on individuals with the G551D mutation in *CFTR*.

Genome-wide studies

As genetic technology has progressed, the more sophisticated tools of genome-wide approaches are now available for studying genetic variation. With the decreasing costs of genotyping assays, it is likely that the genome-wide approach will supersede the candidate approach. However, due to statistical probabilities, it is possible that thousands of SNPs will be falsely associated with specific clinical phenotypes. Although the use of a much lower *P* value than the commonly accepted value of 0.05 can reduce the number of falsely positive SNPs that are identified; however, detailed evaluations of identified SNPs will be essential.

Evaluations must include serious discussion of plausible biological mechanisms, linkage studies, haplotype studies, and replications in independently collected populations, in order to determine which statistically significant SNPs are truly biologically significant.

In 2007, three North American groups studying genetic modifiers in CF formed a consortium to sequence the genomes of participants within their study populations (each numbering over a thousand participants) and to pool the resulting information. It is hoped that such an effort with such detailed genetic information and increased power through collaboration will lead to new insights concerning genetic modifiers into CF. With this information in hand, the subsequent challenge will be translating these discoveries to continue the fight to decrease the morbidity and mortality of this devastating disorder.

Conclusion

Although therapeutic advancements have led to decreased morbidity and improved survival in individuals with CF, the median predicted age of survival in 2006 is approximately half that of the general population. Further advances in care will come from understanding the genetic and environmental modifiers of CF disease to target existing therapies more efficiently and to develop novel therapies based on newly discovered modifiers. Recent studies have revealed several genetic modifiers and imminent genome-wide studies are likely to uncover additional genes that could be targets for therapy for this life-limiting disease.

References

References and recommended reading

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• of special interest

•• of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 000−000).

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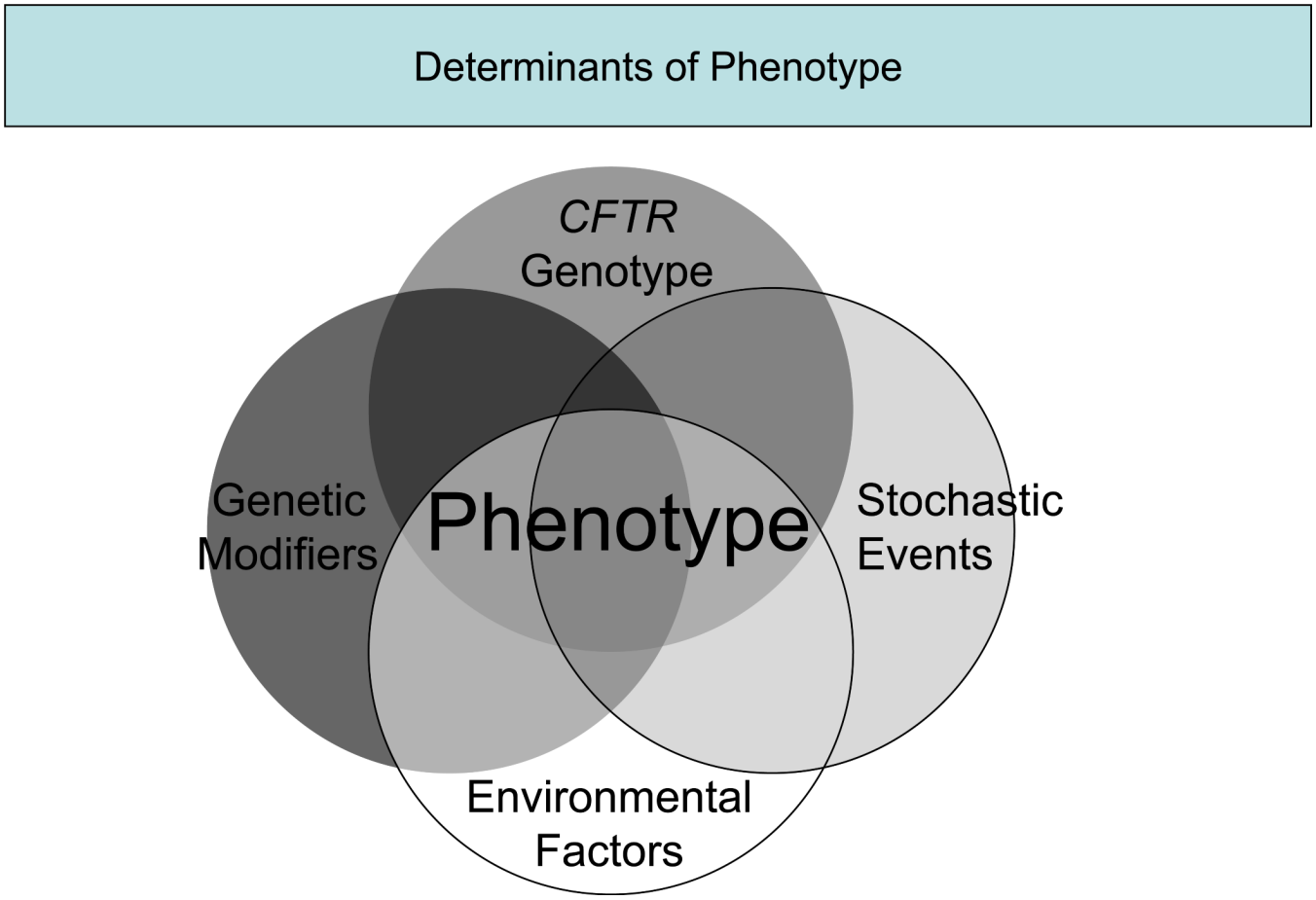

Figure 1.
Multiple factors interact to determine phenotype gr1

Table 1

Summary of replicated findings in candidate gene association studies

Gene	Lung function	Infection Acquisition/Prevalence	Nutritional Status	Survival
TGFB1	Probable effect	No effect		Likely no effect
MBL2	Probable effect	Possible effect		
TNFα	No effect	Likely no effect	Likely no effect	Likely no effect
SERPINA1	No effect	Likely no effect		
GSTM1	No effect			
GSTP1	No effect			
ADRB2	No effect			

Probable effect: association observed in three or more independent studies with ≥1000 participants in aggregate.

Possible effect: association observed in two or more independent studies with ≥500 participants in aggregate.

Likely no effect: no association observed in two or more independent studies with ≥500 participants in aggregate.

No effect: no association observed in three or more independent studies with ≥1000 participants in aggregate.