JCI The Journal of Clinical Investigation

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J Clin Invest. 2008;118(3):839-841. https://doi.org/10.1172/JCI35138.

Commentary

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Gene modifiers in cystic fibrosis

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Phenotypic variability and the search for modifier genes in cystic fibrosis

Cystic fibrosis (CF) is a complex condition affecting a number of organs, including the exocrine pancreas, intestine, sweat glands, and lung; the effects of the latter are the major cause of disease morbidity and early mortality. Identification of the CF transmembrane conductance regulator (*CFTR*) gene in 1989 (1) was followed by the discovery of hundreds of mutant *CFTR* alleles and by attempts at genotype-phenotype correlations (2). The associations between *CFTR* genotype and exocrine pancreatic and sweat gland phenotypes were soon found to be greater than the association between *CFTR* genotype and the pulmonary phenotype (2, 3). Pulmonary phenotype is widely variable, even among patients with the same *CFTR* genotype. This variability could arise from some combination of environmental, stochastic, and modifier gene effects. Studies of twins and of siblings, which control for CF genotype and environment, have demonstrated high degrees of heritability of pulmonary outcomes (4), intestinal obstruction at birth (5), and early exocrine pancreatic dysfunction (6), supporting an important role for modifiers in explaining clinical variability of this disease.

Nonstandard abbreviations used: CF, cystic fibrosis; CFTR, CF transmembrane conductance regulator; MBL, mannose-binding lectin.

Conflict of interest: The authors have declared that no conflict of interest exists.

Citation for this article: *J. Clin. Invest.* **118**:839–841 (2008). doi:10.1172/JCI35138.

Identification of modifier genes could be useful in several ways. Modifiers could provide clues to disease pathogenesis that might lead to development of new treatments. In addition, knowledge of modifiers might improve stratification for clinical trials and clinical prognostication. For these reasons, several groups began modifier studies. At least one validated modifier of lung disease in CF has been identified. A large study, primarily in adults, examining extremes of pulmonary CF phenotypes found that a genetic variant leading to higher production of TGF-β is associated with worse pulmonary outcome (7). More than 20 other candidate modifiers have been examined, often in relatively small studies and frequently without validating populations, leading to conflicting results (8).

In this issue of the *JCI*, Dorfman et al. demonstrate in their large, prospective study that mannose-binding lectin 2 (MBL2), a protein key to the innate immune response, is a modifier of CF lung disease in children and adolescents: individuals possessing genetic variants that result in low MBL2 production had an increased rate of decline of lung function (9). This was first proposed in 1999 (10), but a large subsequent study focusing on older CF patients was unable to confirm the findings (7). In addition, Dorfman et al. found that MBL2 is associated with earlier *Pseudomonas aeruginosa* colonization. They further demonstrate that TGF-β1, when considered alone, is a modifier of lung function decline in CF but not of age at *P. aeruginosa* colonization in CF. To make matters more interesting, they show statistically significant gene-gene interaction between *MBL2* and *TGFB1* in decline in lung function and age at *P. aeruginosa* colonization. This is believed to be the first demonstration of modifier gene-gene interaction in CF.

Several aspects of their experimental approach and additional findings are noteworthy (9). The Canadian CF Modifier Study, from which the patients investigated by Dorfman et al. were recruited, offers the largest number of patients yet for this area of research. Because many clinics and research centers participate, thus providing a patient population representative of the overall CF population, it is likely that the findings are robust. The investigators restricted their study population to individuals with insufficiency of the exocrine pancreas — a condition, manifest in 85% of patients with CF, in which the individual cannot properly digest food due to the absence of digestive enzymes made by the pancreas. In general, these patients have worse outcome than patients with sufficiency of the exocrine pancreas, emphasizing the need for careful investigation of this insufficient group. MBL2 protein levels were determined to confirm the effects of genetic variation — yet another strength of the article. However, TGF-β1 protein levels were not determined, possibly because of difficulties with this assay. Two important pulmonary end points were chosen: age at detection of first *P. aeruginosa* infection (the most common respiratory pathogen in CF patients) and rate of decline in lung function. The latter was assessed through forced expiratory volume in 1 second, which is the best predictor of pulmonary outcome in CF. The choice of end points is meaningful because delaying *P. aeruginosa* infection and slowing the rate of decline in lung function are currently the key goals of CF treatment (11, 12). The magnitude of the modifier effects, namely the delayed age of onset of *P. aeruginosa* infection by several years and differences in decline of lung function of 1%–2% per year, would be considered clinically very meaningful and likely would translate into differences in survival. A very interesting additional

finding in the study of Dorfman et al. is the remarkable similarity in clinical characteristics between patients homozygous for the ΔF508 CFTR mutation in CF and patients who carry 2 different exocrine pancreatic-insufficient mutations with or without ΔF508 (9). Based on in vitro and in vivo studies, it is currently believed that exocrine pancreatic-insufficient patients have 1% or less of CFTR function compared with their wild-type counterparts (13). The findings of Dorfman et al. (9) tell us that the pathophysiology accompanying exocrine pancreatic insufficient mutations is essentially the same, regardless of the type of mutation.

MBL2, TGF-β1, and current models of CF pathophysiology

Current models of CF airway pathophysiology include elements of impaired ion transport leading to abnormal bacterial clearance, persistent infection, intense inflammation, and structural injury. Both MBL2 and TGF-β1 could easily contribute to this cascade. Patients in other clinical settings who have genetic variants of *MBL2* that result in low MBL2 protein levels are at risk for a variety of bacterial, viral, and fungal infections, including pathogens commonly encountered in CF such as *P. aeruginosa*, *Staphylococcus aureus*, and *Hemophilus influenzae*. As has been pointed out, low MBL levels in CF could make it just that much easier for offending bacteria to take hold in an airway that is already compromised (10). There is also some evidence that low MBL–producing genetic variants are associated with early mortality in CF (14, 15). Studies of MBL supplementation in CF may be justified, perhaps not only in patients who have low MBL levels.

The TGF-β signaling pathway is important in numerous human disease states, including more than a dozen hereditary conditions and almost as many multifactorial diseases (15). Many of these entities involve connective tissue formation or fibrosis in some manner. Very little is known about the mechanisms that lead to the prominent fibrosis and bronchiectasis that characterizes the structural injury to the CF airway. It is tempting to speculate that high TGF-β expression is associated with worse pulmonary outcome in CF because of accelerated airway scarring. TGF-β can also have both pro- and anti-inflammatory activities. However, this is dependent on the site, cells involved, and stimuli (15). Given the important role of inflammation in CF, TGF-β could also be acting in this limb of the pathophysiologic schema.

Are there more modifiers to be discovered?

Some notion of whether there are remaining modifiers to be discovered can be gained by comparing the effects of the known modifiers to estimated heritability. The magnitude of the effects of TGF-β1 and MBL2 observed by Dorfman et al. (9) is modest in terms of the number of patients affected. Roughly 20% of patients have either an MBL2 variant or a TGF-β1 variant that contributes to poorer outcome (9). Given the high degree of heritability of pulmonary outcomes, which approached a correlation coefficient of 0.8 in identical twins (4), it is almost certain that important modifiers remain unidentified. Future studies are therefore likely to identify other genes that play a role in modulating disease severity — some independently of others, but many involving gene-gene interactions. Each modifier is likely to have a modest effect, and as the most promising genes are validated or refuted, the remaining candidates may demonstrate an even smaller overall effect or may be influential in a smaller number of patients. To test each candidate and the interactions between them will require many more patients. These large-scale investigations will not be able to be carried out by any one of the existing gene modifier studies.

In a sense, the search for gene modifiers in CF is similar to the search for complex traits in polygenic diseases such as diabetes or hypertension. The effects of individual modifiers in monogenic conditions, as with individual traits in polygenic diseases, may be relatively small, but the overall picture becomes clearer as gene upon gene is identified through even larger collaborative efforts.

CF care and research are moving targets

CF care and research are changing. Many more therapeutic agents are undergoing clinical trials (16). Response to a given treatment could rely on modifier status, which suggests that gathering this information should be part of clinical trial design. In addition, early treatment of *P. aeruginosa* infection is changing the prevalence of this microbe (12). Will current modifier effects defined in terms of *P. aeruginosa* infection persist if the prevalence of this organism changes? It is also likely that in the future, parents will demand all the prognostic information available. Are we at the point where parents should be given information on the modifier status of their child? Dorfman et al. (9) advise caution with respect to use of their results for stratification in clinical trials or in counseling parents. They believe that careful quantitation of sensitivity and specificity of modifier effects should be obtained by prospective studies of infants identified through newborn screening before we change current trial stratification or parental counseling approaches. Indeed, most communities are moving rapidly toward newborn screening and early diagnosis because of demonstrable clinical benefit (17). The collective work of Dorfman et al. and others in the modifier field should inform the longitudinal studies of early treatment of CF that are almost certain to follow adoption of universal newborn CF screening.

Acknowledgments

This work was supported in part by Cystic Fibrosis Foundation grant C017 and National Heart, Lung, and Blood Institute grant 1 U01 HL081335-01.

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Hepatic glucose sensing: does flux matter?

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In this issue of the *JCI*, Denechaud et al. report studies investigating the role of the liver X receptors (LXRs) LXRα and LXRβ in carbohydrate sensing by the liver (see the related article beginning on page 956). The results of this study, which utilized LXRα/β double-KO mice, strongly contradict a recent *Nature* report that proposed that LXRα/β sense glucose independent of metabolic flux. The reported findings further support a key role for the carbohydrate-responsive element–binding protein (ChREBP) in the regulation of lipogenic genes by glucose and dietary carbohydrates.

The liver plays a central and vitally important role in glucose homeostasis by continuously adapting its own metabolism to the availability of nutrients and overall energetic needs of the body. The conversion of carbohydrates and amino acids into fatty acids, a process referred to as lipogenesis, is important for the preservation of excess energy. This metabolic conversion involves the induction of a set of lipogenic genes that have long been known to be regulated by dietary intake, particularly carbohydrates. However, because many lipogenic genes are also regulated by insulin and the concentration of insulin itself is acutely sensitive to changes in blood glucose concentration, only in recent years have we learned how carbohydrate, principally glucose, is able to modulate gene expression in a cell-autonomous manner.

The ability of mammalian cells to sense changes in glucose has long been thought to depend on changes in metabolic flux, as determined by the specific hexose transporters and hexokinases expressed in a given cell. Hepatocytes express both glucose transporter 2 (GLUT2) and glucokinase (GK; also known as hexokinase 4), both of which exhibit affinities for glucose (K_m and glucose $S_{0.5}$, respectively) in the low millimolar range. This enables metabolic flux in hepatocytes to essentially parallel any change in blood glucose concentration (1). In this regard, the liver mimics the pancreatic β cell, the prototypical glucose-sensitive cell type. However, functional parallels between these 2 cell types begin to fade beyond this point, due to the very distinct physiological functions of the liver and β cell in maintaining glucose homeostasis.

While mammals are thought to sense glucose in a manner that directly reflects glucose flux, other mechanisms that do not rely on catalysis exist in both plants and yeast. For instance, the plant hexokinase HXK1 remains capable of signaling after being mutated in a manner that eliminates all catalytic activity (2). A recent study in *Nature* by Mitro et al. reported that glucose is able to directly bind to and increase the transcriptional activity of the liver X receptors (LXRs) (3), thereby suggesting that these nuclear receptors also function as glucose sensors in the liver. If true, this would establish a new paradigm for glucose sensing in mammalian cells. The study by Denechaud et al. in this issue of the *JCI* (4) was directed at exploring this proposed paradigm by assessing the effects of a high-carbohydrate diet on hepatic lipogenesis in mice lacking both LXRα and LXRβ.

SREBP-1c and ChREBP: key players in hepatic lipogenesis

Over recent years, information has emerged indicating that SREBP-1c and carbohydrate-responsive element–binding protein (ChREBP) both contribute to the regulation of lipogenesis in liver, albeit in very distinct ways. SREBP-1c, the SREBP isoform expressed at high levels in the liver (5), is a member of the basic helix-loop-helix (bHLH) leucine zipper family of transcription factors. This family of transcription factors was originally identified through its role in regulating cholesterol and fatty acid biosynthesis by sterols (6, 7). However, SREBP-1c was later found to be regulated by insulin signaling (8). SREBP-1c affects the expression of target genes by binding to sterol response elements, usually located near the promoter region of a target gene. In addition to the regulation of SREBP-1c gene expression by insulin, insulin appears to regulate the cleavage of SREBP-1c, which also directly affects the amount of SREBP-1c in the nucleus (9). SREBP-1 KO mice exhibit profound abnormalities in lipid metabolism (10).

ChREBP, another bHLH transcription factor, also plays a central role in the dietary regulation of hepatic gene expression. This factor was identified by its binding to carbohydrate response elements (ChOREs) in a variety of glucose-respon-

Nonstandard abbreviations used: ACC, acetyl-CoA carboxylase; ChORE, carbohydrate response element; ChREBP, carbohydrate-responsive element–binding protein; FAS, fatty acid synthase; GK, glucokinase; G-6-P, glucose-6-phosphate; L-PK, liver–pyruvate kinase; LXR, liver X receptor.

Conflict of interest: The authors have declared that no conflict of interest exists.

Citation for this article: *J. Clin. Invest.* **118**:841–844 (2008). doi:10.1172/JCI35137.