LEARNING OBJECTIVES

After completing this chapter, the reader should be able to

- Describe the genetic variants that can affect the response to treatment with β₂-agonists, inhaled corticosteroids, leukotriene modifiers, and theophylline in asthmatic patients.
- Discuss the pros and cons of genetic testing to guide asthma therapy.
- Explain the role of the cystic fibrosis transmembrane conductance regulator (CFTR) in the pathophysiology of pulmonary disease in cystic fibrosis.
- Describe the various classes of mutations found in CFTR and their effect on CFTR expression and function.
- Differentiate between therapeutic agents that help manage cystic fibrosis symptoms and disease-modifying agents that modulate CFTR activity.
- Describe how genetic testing is used to identify cystic fibrosis patients who will respond to Kalydeco (ivacaftor) and Orkambi (lumacaftor/ivacaftor).

KEY DEFINITIONS

ALLELE—one of a pair of genes on a specific location of a chromosome that controls the same trait.

BINDING MOTIF—a short, highly conserved region in a protein sequence that is involved in binding.

BIOFILM—structural layer of microorganisms adhered to a surface and held together and protected by a polymeric matrix formed from secreted or extracellular substances.

BRONCHIECTASIS—localized and irreversible dilation of the bronchiole or bronchi due to airway inflammation and obstruction.

CHEMOATTRACTANT—inorganic or organic substance that induces or influences cell migration.

FORCED EXPIRATORY VOLUME IN 1 SECOND (FEV₁)—the volume of air that can be forced out in the first second after taking a deep breath.

INTERPATIENT VARIABILITY—differences in response to drug(s) between patients.

ISOFLAVONE—naturally occurring organic compounds derived almost exclusively from the bean family.

Note: Significant contributions from Dr. Emily Weidman-Evans and Dr. W. Greg Leader as authors of earlier versions of this chapter, published in the previous edition, are gratefully acknowledged.
NASAL POTENTIAL DIFFERENCE (NPD)—a measurement of the voltage across the nasal epithelial cell determined by using a high impedance voltmeter between two electrodes, one placed on the inside and one on the outside of the epithelia. The nasal potential difference characterizes the voltage created by the secretion or absorption of chloride ions that are abnormal in cystic fibrosis.

OPSONOPHAGOCYTOSIS—the process by which a foreign body (usually microbial) is identified by complement or antigens to phagocytes, resulting in a more efficient energy dependent endocytosis (or engulfment) of the microbe by the phagocytic cell (usually a neutrophil or macrophage).

PEAK EXPIRATORY FLOW (PEF)—the maximum airflow during a forced expiration beginning with the lungs fully inflated.

UBIQUITINATION—degradation of a protein caused by modification of the protein by covalent attachment of ubiquitin, a regulatory protein, which directs the protein to proteasomes for degradation.

INTRODUCTION

Asthma and cystic fibrosis (CF) are two very different conditions that have major effects on the respiratory system. Asthma is characterized by chronic inflammation of the airways and bronchoconstriction, resulting from both multiple genetic and environmental factors. CF is a genetic condition that also affects other organ systems, caused by a mutation in the gene encoding for a single ion channel. In light of the differences between the two conditions, the pharmacogenomic research that has been applied to the conditions also differs. In asthma, interpatient variability in response to the available treatments has led to retrospective pharmacogenetic analyses to identify genetic differences affecting responses to currently accepted therapy. The identification of genetic variants that can impact the efficacy and safety of treatment might then allow for rational changes in drug therapy before an exacerbation or adverse effect occurs. In contrast, with CF, genetic mutations that affect the severity of the disease have been identified, and new therapies are being prospectively investigated that will address the physiologic effects of the mutations at the level of the affected protein. This research has resulted in the development and approval of two mutation-specific therapies for CF. In spite of the differences in approach, the end result of the pharmacogenomic research in both of these conditions is improved patient health and clinical outcomes, which is the ultimate goal of all healthcare providers.

CASE STUDY—ASTHMA

R.P. is an 8-year-old African-American male admitted to the emergency room for acute shortness of breath and wheezing. He is diagnosed with an asthma exacerbation, his third episode in the past 12 months. His current medications include fluticasone/salmeterol (dry powder inhaler) 500 mcg/50 mcg twice daily, montelukast 5 mg daily, and albuterol (metered-dose inhaler) 1–2 puffs as needed. At his last admission, he was sent home with a 10-day course of prednisone and started on montelukast; his dose of fluticasone/salmeterol was increased from 250 mcg/50 mcg. In the 24 hours prior to this admission, R.P.’s mother states that he probably used his albuterol 10 times, and that it “just didn’t
seem to work.” Furthermore, you note that his FEV₁ is currently only 50% of expected, and that it is not reversing with the administration of albuterol via nebulizer.

**Questions**

1. Polymorphisms found in the β₂AR gene can influence how an individual responds to β₂-agonists. What will be the likely effect of a Thr164Ile polymorphism on how an individual responds to salmeterol?

2. The presence of which single nucleotide polymorphism (SNP) in the promoter region of the LTA4H gene could result in an asthma patient responding poorly to treatment with montelukast?

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**CASE STUDY—CYSTIC FIBROSIS**

A 5-month-old infant girl Aria is brought to an emergency room for a rapidly spreading diaper rash and chronic cough according to her parents. The attending pediatrician notices salt crystals on Aria’s skin and suspects that the baby could be suffering from CF. A sweat test is ordered, and the results confirm that Aria suffers from CF. Genotyping tests are conducted, and they show that Aria is homozygous for the ∆F508 mutation.

**Questions**

1. Is Aria likely to be prescribed ivacaftor for her condition?

2. If pancreatic enzyme supplementation is prescribed for Aria, will pancreatic enzyme replacement help correct the underlying genetic defect that causes CF?

3. Can any therapeutic options available for Aria help modulate the activity of CFTR?

4. A new investigational drug that acts as a potentiator is shown to have positive effects in cells and animals with Class III mutations only. Is Aria likely to benefit from this drug if it is approved for treating CF?

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**ASTHMA**

**Introduction**

Asthma is a chronic inflammatory airway disease. In the United States, asthma afflicts over 22 million people, of which more than 6 million are children, making it one of the most common chronic childhood diseases.¹ The natural history of asthma, though variable, is classically characterized by wheezing, cough, and shortness of breath. Patients with asthma typically exhibit episodic symptoms that occur following exposure to specific triggers like allergens, exercise, or cold air.

The foundation of asthma pathophysiology is inflammation. In studies of allergic asthma, inhaled allergens are encountered in the airways by antigen-presenting cells of the immune system resulting in the production of cytokines and allergen-specific IgE, which attaches to mast cells in the airways. Airway mast cells exposed to a trigger rapidly degranulate releasing histamine, leukotrienes, and cytokines that cause bronchospasm, edema, and airflow obstruction leading to asthma symptoms. Approximately 6 to 9 hours after allergen incitement, in the late phase reaction, eosinophils, T lymphocytes, basophils, neutrophils, and macrophages are activated resulting in enhanced inflammation and bronchial hyperresponsiveness.²

Asthma therapies seek to attenuate this inflammatory response and subsequent physiologic events like bronchospasm. Inhaled corticosteroids (ICS), leukotriene (LT) modi-
fiers, and an anti-IgE antibody target the inflammatory response. Bronchospasm is targeted by theophylline and $\beta_2$-receptor agonists, which can be divided into short-acting agonists (SABA) and long-acting agonists (LABA).

Pharmacotherapy of asthma is applied in a stepwise fashion and is predicated on a patient’s age, disease severity, and response to therapy. At initial diagnosis, disease severity is assessed with spirometry, symptom frequency and timing, and interference with daily activities. Appropriate therapy is initiated based on severity classification. As outlined in Table 11-1, all patients with asthma receive a SABA and, in addition, may receive daily maintenance medication depending on severity classification. Therapy may be stepped up or down based on clinical response.

**Genetics**

It has long been established that there is a genetic link in asthma, involving interactions of multiple genes and a variety of environmental factors, including allergens and infections. Independent genome-wide screens have found regions of linkage with asthma susceptibility and severity on chromosomes 5, 6, 11, 12, 13, 16, 17, and 19. It is only logical, then, to begin pharmacogenomic research based on differences in these genetic regions.

In spite of the well-accepted treatment guidelines for this condition, a large degree of interpatient variability in treatment response was noted in drug studies. Fortunately, we do have a relatively good grasp on the precise mechanisms of a number of drugs that are being used to treat asthma. Therefore, identifying potential genetic targets that involve the receptors or mediators affected by the drugs is the most straightforward approach. To date, specific genes have been identified that affect bronchodilation directly, the production and action of drug receptors, and a number of steps in the inflammatory process (e.g., T-cell proliferation and recruitment, macrophage recruitment, mast cell proliferation.

<table>
<thead>
<tr>
<th>Severity Classification</th>
<th>Treatment Step</th>
<th>Preferred Therapy</th>
<th>Alternative Therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intermittent</td>
<td>Step 1</td>
<td>SABA as needed</td>
<td></td>
</tr>
<tr>
<td>Mild persistent</td>
<td>Step 2</td>
<td>Low-dose ICS</td>
<td>Cromolyn, LTRA or theophylline</td>
</tr>
<tr>
<td>Moderate persistent</td>
<td>Step 3</td>
<td>Low-dose ICS + LABA or medium-dose ICS</td>
<td>Low-dose ICS + either LTRA, theophylline or zileuton</td>
</tr>
<tr>
<td>Severe persistent</td>
<td>Step 4</td>
<td>Medium-dose ICS + LABA</td>
<td>Medium-dose ICS + either LTRA, theophylline or zileuton</td>
</tr>
<tr>
<td>Severe persistent</td>
<td>Step 5</td>
<td>High-dose ICS + LABA</td>
<td>Consider omalizumab for patients who have allergies</td>
</tr>
<tr>
<td>Severe persistent</td>
<td>Step 6</td>
<td>High-dose ICS + LABA + oral corticosteroid</td>
<td>Consider omalizumab for patients who have allergies</td>
</tr>
</tbody>
</table>

ICS, inhaled corticosteroid; LABA, long-acting beta-2 receptor agonist; LTRA, leukotriene receptor antagonist; SABA, short-acting beta-2 agonist.
antigen presentation, inhibition of cytokine activity). The genetic variances that impact four common classes of drugs used in asthma are presented below.

**β₂-Agonists**

Beta-2 adrenergic receptor (β₂AR) agonists are agents that bind to and activate β₂ adrenergic receptors. The β₂AR is a 413 amino acid protein that belongs to the G-protein coupled receptor family and consists of seven transmembrane segments. β₂ARs are

- widely distributed throughout the body including cardiac, respiratory, and endocrine tissues.
- administered via the inhalational route to limit their effects to the respiratory tract.

Their activation in bronchial smooth muscle stimulates cyclic adenosine monophosphate (cAMP) production, which leads to a decrease in intracellular calcium and subsequent smooth muscle relaxation and bronchodilation.

β₂-agonists are divided into two distinct clinical categories based on duration of action and consequent clinical use: short-acting and long-acting β₂-agonists (SABAs and LABAs). SABAs, including albuterol, levalbuterol, and pirbuterol, provide immediate, short-term relief of asthma symptoms. SABAs are prescribed to all patients with asthma, regardless of severity and used on an as-needed basis for control of acute symptoms. LABAs, including salmeterol and formoterol, provide long-term relief of asthma symptoms. LABAs are dosed twice daily as maintenance therapy and are indicated for patients with moderate-to-severe disease in addition to an inhaled corticosteroid.

**Interpatient Variability**

The most clinically relevant concern regarding β₂-agonists is the increased risk of life-threatening and fatal asthma exacerbations with LABA use, prompting a black box warning from the U.S. Food and Drug Administration (FDA). Recognition of this risk occurred after a postmarketing study of nearly 25,000 patients showed an increased absolute number of deaths in patients receiving salmeterol versus albuterol in addition to usual asthma therapy. These findings, however, were not statistically significant and follow-up studies could not reproduce this risk. Therefore, a large trial assessing the safety of salmeterol, the Salmeterol Multicenter Asthma Research Trial (SMART), was undertaken and an interim analysis showed an increase in asthma-related death, particularly in African Americans. A subsequent meta-analysis including these patients corroborated these findings. Despite these findings, the benefit of LABA therapy, particularly with concurrent inhaled corticosteroid therapy, was deemed to merit continued recommendation of LABAs for asthma. It has been postulated that concurrent therapy with an inhaled corticosteroid may obviate this risk, although definitive studies are lacking.

**CLINICAL PEARL**

It was theorized at the time that the disparity seen in African Americans in the SMART trial was due to either more severe underlying disease and/or the fact that the baseline ICS use in this subpopulation was lower.
**Genetic Variants Affecting Treatment**

The high degree of interpatient variability in response to \( \beta_2 \)-agonists, as well as the possible relationship between treatment and race exhibited in SMART, leads to questions regarding a possible genetic cause of the variability. Table 11-2 shows the three nonsynonymous coding region SNPs in the \( \beta_2 \)-AR gene, which is located on chromosome 5q31-32, that could contribute to some of the demonstrated differences in individual responses to \( \beta_2 \)-agonists in asthma and other respiratory diseases.\(^{12,13}\) These SNPs are substitutions of glycine for arginine at the 16th amino acid (Arg16Gly), of glutamate for glutamine at the 27th amino acid (Gln27Glu), and of isoleucine for threonine at the 164th amino acid (Thr164Ile). In addition to these three SNPs on the \( \text{ADRB2} \) gene, polymorphisms on a few other genes have also been identified that may influence the response \( \beta_2 \)-agonists. All of these polymorphisms occur with the same frequency in both asthmatic and nonasthmatic patients, and thus none of them can be used for predicting risk for asthma.\(^{12,14}\) Genome-wide association studies (GWAS) have also not identified an association between polymorphisms on the \( \beta_2 \)-AR gene and risk for asthma.\(^{15}\)

**ARG16GLY (285 A>G).** Three meta-analyses have shown that this relatively common polymorphism is not associated with asthma diagnosis. However, some evidence suggests that this polymorphism may be associated with nocturnal asthma and a more severe asthma phenotype.\(^{17,18}\) Several studies have suggested an association between the Arg16Gly polymorphism and response to SABA.\(^{19-23}\) Individuals homozygous for Arg-16 have been shown to have a greater and more rapid response to albuterol treatment than individuals who are homozygous for Gly-16.\(^{19,20}\) Other studies have shown that patients homozygous for Arg-16 who receive a regularly scheduled SABA long-term experience decreases in peak expiratory flow readings (PEFR) and forced expiratory volume (FEV). This patient population also exhibited a worsening of daily symptoms, increases in the amount of rescue medication required, and an increased number of exacerbations.\(^{21-23}\) In clinical practice, patients who require SABAs consistently throughout the day would generally have additions, intensifications, or modifications to their maintenance asthma therapy medications. These studies suggest that regular SABA therapy may necessitate more timely maintenance medication adjustment in patients who are homozygotes of Arg-16.

### Table 11-2

<table>
<thead>
<tr>
<th>SNP</th>
<th>Frequency of (Homozygotes)(^{14,16,17,130,131})</th>
<th>Response to ( \beta_2 )-Agonists(^{12,14,26})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arg16Gly</td>
<td>Overall: 54.8–60.4 White: 60.7 Asian: 46.0 Black: 45.0</td>
<td>Fewer exacerbations and reduced need for rescue medication for patients receiving regular SABA therapy</td>
</tr>
<tr>
<td>Gln27Glu</td>
<td>Overall: 24.7–47.2 White: 36.1 Asian: 13.6 Black: 18.0</td>
<td>Reduced risk of developing tachyphylaxis</td>
</tr>
<tr>
<td>Thr164Ile</td>
<td>Overall: 5.0</td>
<td>Decreased binding of drug to receptor → possible decrease in drug activity</td>
</tr>
</tbody>
</table>

SABA = short-acting beta-2 agonist, SNPs, single nucleotide polymorphisms.
Due to the relationship between genotype and response to SABAs, as well as the controversy surrounding the use of LABAs, several studies have been conducted to determine the relationship between LABAs and the Gly-16 SNP. Unfortunately, the results of these studies are conflicting. Bleeker and colleagues conducted a pharmacogenetic analysis of two trials that used either salmeterol or formoterol, which showed no difference between genotypes in exacerbations, spirometric measures (PEFR and FEV₁), or adverse effects. All of the subjects in this analysis received ICS in addition to a LABA. Conversely, a similar analysis conducted by Weschler and colleagues of two large studies utilizing salmeterol showed that those subjects who were homozygous for the wild-type receptor had lower spirometric measures as well as increased symptoms and albuterol use regardless of concurrent ICS use. Those homozygous for Gly-16 showed an improvement in spirometric and symptomatic measures during the study periods. A retrospective study in 546 children and young asthmatics showed a two-fold increase in exacerbations in those with Arg-16. In patients receiving salmeterol, the risk increased to over three times that of those with Gly-16.

**CLINICAL PEARL**

An association has been established between the Arg16Gly polymorphism and response to β₂-agonists. However, the effects of this polymorphism depend on whether LABAs or SABAs are used, and whether SABAs are used long-term on a regular or an as-needed basis.

GLN27GLU (318C>G). Some studies have shown an association between the Glu-27 polymorphism and a protective effect against bronchial hyperresponsiveness and severity of asthma. Glu-27 may also afford protection against tachyphylaxis to β₂-agonist therapy. A study involving 80 Spanish asthmatic patients showed that patients with Gln-27 were more likely to experience tachyphylaxis than patients with the Glu-27 polymorphism.

THR164ILE (730C>T). The Ile-164 is a rare polymorphism compared to both Arg16Gly and Gln27Glu. In vitro studies have suggested that the activity of the receptors displaying this polymorphism is about half that of wild-type receptors. The binding affinities for albuterol, terbutaline, formoterol, and salmeterol were all found to be lower (Kᵢᵢjs being 12–3.0 fold higher) for Thr164Ile when studied in transfected fibroblasts. Furthermore, studies in transgenic mice showed a decreased cardiac response to isoproterenol when compared to mice with the wild-type receptor, leading to the hypothesis that those with this polymorphism could also have a lower baseline bronchodilator response to inhaled β₂-agonists.

**OTHER VARIANTS AFFECTING RESPONSE TO β₂-AGONISTS.** Candidate gene studies and GWAS have resulted in identification of polymorphisms on other genes within B₂AR signaling pathways that influence response to β₂-agonists. Polymorphisms within the CRHR2, ADCY9, ARG1, ARG2, NOS3, THR2, SLC24A4, SLC22A15, and SPATS2L genes have been associated with alterations in the therapeutic response to β₂-agonists. In a study involving asthmatic children, it was found that the Ile772Met polymorphism on the ADCY9 gene contributed to an enhanced bronchodilator response to albuterol in children that were also receiving budesonide. Polymorphisms within ARG1, ARG2, and NOS3 can influence the production of the endogenous bronchodilator nitric oxide and thereby influence response.
to $\beta_2$-agonists. GWAS have identified a polymorphism in the promoter region of $SPATS2L$. This study also highlights the potential of GWAS in identifying novel genes that may be involved in regulating the response to $\beta_2$-agonists. Polymorphisms found in genes coding for the solute carrier group of ion transporters have also been found to influence bronchodilator response. These studies highlight the polygenic nature of asthma in that a variety of different polymorphisms found in different genes can influence the response to $\beta_2$-agonists. Additional studies will be required to better define the role and importance of each of these genes as well as the combined effect of polymorphisms in these genes on bronchodilator response.

**Inhaled Corticosteroids**

ICS are the most potent and effective medications for long-term control of asthma symptoms. As such, the majority of patients with asthma receive an ICS as part of their treatment regimen. The ICS exert their pharmacologic activity by binding to intracellular glucocorticoid (GC) receptors. Activation of GC receptors alters gene transcription and may also affect post-translational events resulting in down-regulation of pro-inflammatory mediators and up-regulation of anti-inflammatory mediators. As with $\beta_2$-agonists, ICS are administered via inhalation to minimize their systemic effects. Available ICS agents include beclomethasone dipropionate, budesonide, ciclesonide, flunisolide, fluticasone propionate, and mometasone furoate. Concerns regarding adverse effects of ICS, including reduced linear growth in children, have been substantiated. However, these risks are balanced by the efficacy of ICS in the control of asthma, provided the lowest effective doses are used.

**Interpatient Variability**

Interpatient variability in response to corticosteroids has been demonstrated in a number of disease states including inflammatory bowel disease (IBD), psoriasis, nephritic syndrome, and various cancers. The term glucocorticoid resistance has been coined to define this variability. In asthma, it has been shown that 5-10% of patients will have a reduced response to ICS. This number increases to 35% in those with severe disease and to nearly 40% in black patients with asthma. A 12-week study showed that 22% of subjects experienced a decrease in their FEV$_1$ of 5% or more with inhaled beclomethasone therapy, while 10% improved by more than 40%. Another study showed 38% of subjects respond minimally to beclomethasone or fluticasone over 24 weeks of therapy. In both of these studies, the average response was approximately a 10% increase in FEV$_1$.

There is less evidence regarding variability in the frequency and severity of adverse effects associated with ICS, most likely because systemic adverse effects are relatively rare. However, one study showed a significant relationship between doses of inhaled triamcinolone, a highly bioavailable ICS, and decreases in bone density. Analysis of individual response showed a large amount of variability in the degree of bone loss experienced between patients who were independent of the number of puffs per day. This finding suggests that factors other than dose were contributing to this adverse effect.

**Genetic Variants Affecting Treatment**

Due to the broad effects of corticosteroids, it is more difficult to pinpoint appropriate genetic targets for focus in pharmacogenomic studies involving ICS. Studies involving genes that code for several key receptors, however, have shown promise in identifying hypo- and hyper-responders to ICS.
CORTICOTROPIN-RELEASING HORMONE RECEPTOR-1. The gene that encodes for corticotropin-releasing hormone receptor-1 (CRHRI) was chosen as a potential target for research because it is the primary receptor mediating the release of adrenocorticotropic hormone (ACTH), a major regulator of glucocorticoid and catecholamine synthesis. This gene, located on chromosome 17q21-22, has been implicated in the pathogenesis of inflammatory diseases and is located in a region linked to asthma in some genome-wide screens. It is hypothesized that alterations in the expression or function of this receptor as a result of genetic variation can lead to decreased ACTH release. This, in turn, will decrease cortisol release in response to inflammation and possibly upregulate glucocorticoid receptors. When patients with this variant are given exogenous corticosteroids, they will likely have a more pronounced therapeutic response.

Variations in CRHRI have been associated with a 2–4 fold increase in response to ICS when compared to those without the variations. One SNP in particular, rs242941, has been linked to a significant increase in ICS response. This SNP, however, is intronic so it does not have any effect on the CRHRI unless it is present in a specific haplotype, designated GAT. This haplotype is present in 27% of whites. A retrospective pharmacogenetic analysis showed that, while all patients on ICS had improved FEV1 after 8 weeks of therapy, GAT homozygotes experienced a two- to three-fold increase in that improvement. Heterozygotes’ response fell in between that of homozygotes and those without the GAT haplotype.

T-BOX EXPRESSED IN T CELLS (TRANSCRIPTION FACTOR T-BET). Transcription factor T-bet plays an important role in the inflammatory process. It influences the development of naive T-lymphocytes, induces the production of interferon-gamma, and represses the production of interleukins 4 and 5. The gene that encodes for T-bet, TBX21, is also located on chromosome 17q21. It has been shown that asthmatic patients have a decreased number of T-cells that express TBX21, and that deletion of this gene in mice results in airway hyperresponsiveness. Furthermore, several mutations of TBX21 have been associated with both asthma susceptibility and severity in humans. The SNP rs2240017, which results in the substitution of glutamine for histidine at the 33rd amino acid (Gln–33), has been linked to a significant increase in response to ICS. A retrospective pharmacogenetic analysis in 701 children showed that those with the SNP had about a three-fold improvement in their PC20 (provocative concentration of histamine causing a 20% fall in FEV1; a measure of airway responsiveness) when compared to those with the wild-type gene. In fact, the mean PC20 for those subjects on ICS with the SNP was 27.7 mg/mL. Anything >25 mg/mL indicates “normal” airway responsiveness. FEV1 increased to a similar extent in all subjects receiving ICS, regardless of the presence of the SNP. The subjects included in this analysis, about 4.5% of the study population, were all heterozygotes for the SNP. The frequency of homozygosity for this beneficial mutation varies greatly based on race: black 0.4% to 2%; white 2.7% to 3.0%; Hispanic 7.1%; and Korean 11.8%.

GLUCOCORTICOID RECEPTOR GENE (GR/NR3C1). Another logical genetic target would be the gene that encodes for the glucocorticoid receptor (GR) itself. This receptor has two naturally occurring isoforms: GRα, which is functional and involved in regulating proinflammatory mediators, and GRβ, which has no hormone-binding activity and is actually considered an endogenous inhibitor of actions mediated by the GR. An imbalance of either of these isoforms due to a genetic anomaly can lead to glucocorticoid resistance. It is known that 95% of patients with glucocorticoid resistance have type I resistance, which is associated with an increased expression of GRβ. The patient will often present with severe
systemic side effects but will have minimal therapeutic effects. Conversely, type II glucocorticoid resistance is associated with a decrease in GRα, which results in a generalized primary cortisol resistance. These patients typically do not experience either therapeutic or adverse effects when administered ICS. However, the specific mutation(s) that result(s) in this imbalance have not yet been identified.

**OTHER VARIANTS AFFECTING RESPONSE TO ICS.** Due to the broad effects of corticosteroids and their interactions with other pathways, additional polymorphisms have also been identified through either candidate gene or GWAS. The heat shock-organizing protein plays an important role in GR hetero-complex association. SNPs on *STIP1*, the gene encoding the heat shock-organizing protein, have been shown to correlate with improvement in lung function following treatment with corticosteroids. CYP3A4*22 allele has been shown to be associated with improved asthma control in children receiving fluticasone propionate (FP). This study suggests that decreased CYP3A4 activity may increase exposure to FP and might help in predicting response to FP. GWAS have identified associations between polymorphisms in novel genes such as the *T* gene and the *GLCCI1* gene and lung function response to corticosteroid treatment.

**Leukotriene Modifiers**

The term *LT modifier* represents two classes of drugs: LT receptor antagonists (LTRAs), which include montelukast and zafirlukast, and the 5-lipoxygenase (ALOX5) inhibitor zileuton. LTRAs directly antagonize the leukotriene receptor CysLT1 preventing endogenous LT binding, while zileuton inhibits the 5-lipoxygenase enzyme responsible for synthesizing L Ts from arachidonic acid. In both cases, LT-mediated signaling is diminished resulting in reduced mucus secretion, airway edema, and bronchospasm.

LT modifiers are clinically effective for the treatment of asthma; however, their efficacy is considered to be inferior to that of ICS and LABAs (in combination with an ICS). Thus, they are recommended as non-preferred alternative therapy (see Table 11-1). However, a distinguishing, potentially attractive, characteristic of these medications compared with those previously discussed is that LT modifiers are administered via the oral, not inhalational, route.

**Interpatient Variability**

Although not as widely studied as β₂-agonists and ICS, one study showed the distribution of responses with montelukast to be similar to that seen with inhaled beclomethasone. Of those receiving montelukast, 42% had an increase in FEV₁ of more than 10%, while 34% of patients had no improvement or a worsening in FEV₁. Because LT appears linked to asthma susceptibility and severity, it is theorized that variations in therapeutic response to this class of drugs are at least partially mediated by the concentration of LT. This suggests that asthma may be mediated by factors other than LT and that LT modifiers will be less effective in those patients with lower LT concentrations. Conversely, higher concentrations of these mediators could indicate a better response to LT modifiers.

Serious adverse effects with the LTRAs are relatively rare, but zileuton has been linked to hepatotoxicity and even rare causes of hepatic failure. One safety surveillance study showed that 4.4% of subjects receiving zileuton (600 mg 4 times daily) had elevations in alanine aminotransferase to greater than 3 times the upper limit of normal; 13% had elevations greater than 8 times the upper limit. Women were more likely than men to
experience these significant elevations, as were the elderly. Although no genetic studies have been performed, it is possible that the genetic mutations discussed below, or others, could contribute to this adverse effect.

**Genetic Variants Affecting Treatment**

Studies have been conducted to determine if the variability in response to LT modifier therapeutics, as described above, is a result of polymorphisms in genes involved in the production of proteins that affect the pharmacodynamics or pharmacokinetics of LT modifiers. Polymorphisms in genes encoding proteins that bind to LTs (CYSLTR1 and CYSLTR2), regulate their production (ALOX5, LTC4S, and LTA4H), or influence disposition of LT modifier drugs (MRP1 and SLCO2B1) have been associated with altered response to LT modifiers. Studies involving each of these classes of proteins are summarized below.

**ARACHIDONATE 5-LIPOXYGENASE GENE (ALOX5).** ALOX5 is involved very early in the LT synthesis process, converting arachidonic acid to 5-hydroperoxyeicosatetraenoic acid (5-HPETE) and then converting 5-HPETE to LTA4.47 The promoter gene for this enzyme, located on chromosome 10q11, contains a regulatory region with five tandem repeats of the binding motif. Table 11-3 shows the frequencies of the variants of this and other enzyme promoter genes in the LT synthesis pathway and highlights the racial differences that exist.50

It has been theorized that certain variant alleles result in a decrease in LT production; therefore, it is likely the airway obstruction experienced in patients with these altered promoter genes is caused by mechanisms other than LT. Patients with these variants will be less responsive to LT modifiers.16 This theory is supported by two studies that showed significant increases in FEV₁ (9.1-18.8%) in subjects homozygous for the wild-type gene, compared to a small decrease (1.1-2.3%) in those with mutations of the gene.16,51 Another

<table>
<thead>
<tr>
<th>Variant Promoter Gene</th>
<th>Homozygotes (%)50</th>
<th>Heterozygotes (%)50</th>
<th>Effect on FEV₁</th>
<th>Effect on Exacerbations50</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALOX5</td>
<td>Overall: &lt;1</td>
<td>Overall: 36</td>
<td>+/-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>White: 3.6</td>
<td>White: 33</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Black: 24.6</td>
<td>Black: 57</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LTC4 synthase</td>
<td>Overall: 11</td>
<td>Overall: 38</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LTA4 hydrolase</td>
<td>Overall: 8</td>
<td>Overall: 44</td>
<td>NE</td>
<td>+</td>
</tr>
</tbody>
</table>

ALOX5, 5-lipoxygenase; FEV₁, forced expiratory volume in 1 second; LT, leukotriene; LTA4, leukotriene A4; LTC4, leukotriene C4.

+, increased.

-, decreased.

+/-, conflicting evidence. NE, not evaluated.
study showed that those homozygous for the polymorphism had no change in FEV₁, β₂-agonist use, or number of asthma exacerbations after 12 weeks of montelukast treatment, while those homozygous and heterozygous for the wild-type gene had significant improvements in each of these parameters. Other research, however, has shown opposite outcomes. One study that compared percent change in FEV₁ and exacerbations with montelukast showed that homozygotes for the variant gene had a fifteen-fold greater increase in FEV₁ when compared to homozygotes for the wild-type gene. In addition, those with at least one mutant allele had a 73% reduction in exacerbations (at least one during the 6-month study period). Obviously, further studies are necessary to clarify this apparent discrepancy.

**LT C4 SYNTHASE PROMOTER GENE (LTC4S).** LTC4 synthase is a late-stage enzyme in the LT production process that is responsible for converting LTA₄ to LTC₄. One specific variant in the promoter gene for this enzyme, SNP A(−444)C (rs730012) located on chromosome 5q35, has been identified in 32% of non-asthmatic patients, 56% of those with severe asthma, and 76% of those with aspirin intolerant asthma (AIA). This variant enhances the expression of LTC4 synthase, thus increasing LTC₄ production. It is hypothesized that since LTC4 and its products contribute so significantly to asthma physiology, those with this SNP would be good responders to LT modifiers. This theory is supported by a small study that involved administering zafirlukast for 2 weeks to 23 subjects with severe asthma and 31 non-asthmatic subjects. Those subjects with at least one variant allele for the promoter gene had a 9% increase in FEV₁, compared to a 12% decrease in those with the wild-type gene. Furthermore, subjects homozygous or heterozygous for the mutant gene had an 80% reduction in exacerbations while taking montelukast. Interestingly, those with at least one variant allele receiving placebo also had a significant decrease in exacerbations (69%) when compared to those homozygous for the wild-type gene.

**LEUKOTRIENE A4 HYDROLASE PROMOTER GENE (LTA4H).** The LTA4H enzyme is responsible for converting LTA₄ to LTB₄. The presence of one SNP (rs2660845) in its promoter gene, located on chromosome 12q22, has been linked with up to a four-fold increase in the risk of patients having at least one exacerbation when taking montelukast. This difference was not observed between genotypes in those receiving placebo. The effect on LTA4 hydrolase activity as a result of this SNP is unknown. It is possible that the increase in exacerbations results from a decrease in the activity of LTA4H, which leads to more LTA4 being converted to LTC4 and increased asthma symptoms. Conversely, the variant could increase the activity of LTA4H, thus decreasing the production of the cys-LT. This would result in a decreased therapeutic response to LT modifiers.

**CYSTEINYL LT RECEPTOR GENES (CYSLTR1 AND CYSLTR2).** Cysteinyl LTs bind to the cysteine LT receptors and mediate the effects correlated with the pathophysiology of asthma. Montelukast, pranlukast, and zafirlukast are LTRAs that bind to the CYSLTR1 receptor and inhibit the effects of cysteinyl LTs mediated through this receptor. A study of 89 AIA patients found that a −634 C>T promoter polymorphism can help predict LTRA requirements in the management of AIA. A study analyzing the association between response to montelukast and polymorphisms in 10 genes found that polymorphisms in CYSLTR2 resulted in a significantly higher improvement in morning peak expiratory flow. These polymorphisms likely predispose individuals to high cysteinyl LT concentrations, thereby causing an enhanced response to LTRA therapy.
SOLUTE CARRIER ORGANIC ANION TRANSPORTER FAMILY MEMBER 2B1 GENE (SLCO2B1). The ability of polymorphisms, in genes encoding proteins that affect LTRA uptake and concentrations, have been investigated. A coding, non-synonymous polymorphism in SLCO2B1 (c. 935G>A) was shown to result in significantly lower morning concentrations of montelukast in patients receiving a dose the previous evening (n = 80 subjects).57 Another study in a relatively smaller population of 16 volunteers showed conflicting results and found no effect of the same polymorphism on montelukast plasma concentrations.58 A third study involving 24 subjects also found no association between the c.935G>A polymorphism and plasma levels of montelukast.59 The conflicting results suggest that SLCO2B1 polymorphisms may only have a minor role in affecting montelukast plasma levels. Additional studies with larger sample sizes would be needed to clearly define the role of SLCO2B1 polymorphisms in influencing plasma concentrations and response to montelukast.

Theophylline

Theophylline is a methylxanthine that exerts its pharmacologic activity by non-selectively inhibiting phosphodiesterase, thereby increasing intracellular cAMP concentrations in various tissues. In respiratory tissue, this causes bronchodilation. In addition, theophylline may also have anti-inflammatory properties.60 Clinical use of theophylline, however, is limited by its extra-respiratory effects including cardiac, gastrointestinal, and central nervous system stimulation. Theophylline has a narrow therapeutic index necessitating routine monitoring of serum drug concentrations and dose adjustments when necessary to maximize efficacy and minimize risk of toxicity.1

Genetic Variants Affecting Treatment

Due to the narrow therapeutic index of theophylline and potential for toxicity, pharmacogenomic studies have focused on genes that ultimately affect serum drug levels.

CYTOCHROME P450 1A2 GENE (CYP1A2). Theophylline is metabolized primarily via demethylation and hydroxylation by hepatic cytochromes P450 (CYP) 1A2, CYP3A3, and CYP2E1. A polymorphism in the 5'-flanking region of the CYP1A2 gene at position −2964 (G>A) has been associated with reduced theophylline clearance.61 This discovery followed initial findings that the same polymorphism was associated with differences in metabolism of a similar methylxanthine, caffeine.62 Based on these data, it has been postulated that patients with the −2964G>A polymorphism in the CYP1A2 gene may require lower doses of theophylline to reduce risk of toxicity.61

Clinical Implications and Testing

The data for each of the genetic variants discussed above demonstrates that response to asthma medication is affected by the presence or absence of certain polymorphisms. Knowledge of specific genetic variants could help healthcare providers individualize therapy and make adjustments before a patient has an exacerbation or adverse response to therapy. There are tests currently available for identifying several of the polymorphisms discussed above, but they are not commonly used in practice. The debate continues regarding the clinical, legal, and ethical implications surrounding widespread genetic testing. The impact of pharmacogenomic testing on clinical decision making, patient privacy, and costs of healthcare is discussed throughout this book. For example, there are proven racial differences in the frequencies of many of the mutations covered here. African Americans are
less likely than whites to have the beneficial variants in $\beta_2$-AR and CRHR1, but they are more likely to have the potentially positive mutation in ALOX5. Could this finding lead to genetic testing prompted by racial background, prior to initiating or altering therapy or in obtaining healthcare insurance?

There is some overlap in genetic polymorphisms with regards to the pathophysiology and susceptibility of asthma with that of both chronic obstructive pulmonary disease (COPD) and allergic rhinitis/atopic dermatitis. This genetic overlap is important because of the many similarities in the treatment of these conditions. As per the 2007 Global Initiative for Chronic Obstructive Lung Disease guidelines, $\beta_2$-agonists and corticosteroids are indicated for a large proportion of patients with COPD. Similarly, the 2008 update of the World Health Organization’s Allergic Rhinitis and its Impact on Asthma guidelines state intranasal corticosteroids are the most effective treatment for allergic rhinitis, and the LT modifier montelukast should be considered in all patients over the age of 6. Although no definitive studies have been done, given these similarities in etiology and treatment, it is likely that the genetic variants identified as having a potential effect on asthma therapy will have similar effects in COPD and allergic rhinitis.

The pharmacogenomic data related to $\beta_2$-agonists, ICS, LT modifiers, and theophylline make it clear that genotyping could help to guide asthma therapy. However, the lack of long-term outcomes studies, the small sample size in many of the studies, and limited replication of the studies, make it hard to advocate for universal genetic screening of asthmatic patients.

**CYSTIC FIBROSIS**

**Introduction**

CF is a hereditary disorder that influences multiple organs and organ systems. CF affects approximately 1 in 3,500 newborns and is the most common autosomal recessive genetic disorder in the United States. In the 1970s, the median survival age of a patient with CF in the United States was in the mid-teens. However, due to significant advances in the diagnosis and treatment of CF, in 2013 the median predicted survival age has increased to >40 years.

CF is caused by mutations in the CFTR gene on chromosome 7 responsible for encoding the CFTR protein. The CFTR protein performs various physiologic functions, including regulation of transmembrane chloride ion transport. On a cellular level, this mutation and the resulting absence of CFTR protein activity is associated with exocrine gland dysfunction causing viscous secretions in organs including the pancreas, intestine, and lungs. This widespread pathology causes a diverse clinical presentation. Pulmonary manifestations may include cough, shortness of breath, and wheezing and are sometimes associated with recurrent respiratory tract infections. Gastrointestinal and pancreatic manifestations may include steatorrhea and malnutrition. Neonates classically present with meconium ileus. CF may also affect the reproductive system causing obstructive azoospermia in males and reduced fertility in females. The diagnosis of CF is based on clinical symptoms or a sibling history of CF that is confirmed through documentation of CFTR dysfunction (Table 11-4).

When CF was initially described, survival past childhood was uncommon. Advances in the diagnosis of CF and the treatment of complications related to CF have dramatically increased the lifespan of patients with the disorder; however, effective methods to correct
the genetic defect, and thus affect a cure, have until recently remained elusive. Despite the fact that CF has been commonly referred to as a pediatric disorder, more and more practitioners who usually deal with adult populations are now caring for CF patients. In 1970 the median predicted survival for a CF patient was 16 years; however, in 2006, the median predicted survival had increased to 36.9 years.73 Adult CF patients are more common today; in 2006, 44.6% of the patients in the Cystic Fibrosis Foundation Registry were 18 years of age or older.73

In 2006, 362 CF deaths were recorded in the CF database sponsored by the Cystic Fibrosis Foundation.74 Over 90% of all CF deaths are related to pulmonary complications of the disease.75

### Pathophysiology

CF is caused by a genetic defect in the production of the CFTR protein, which is a member of the adenosine triphosphate (ATP)-binding cassette transporter ATPases. CFTR is a 1480 amino acid glycoprotein that functions as a cAMP-regulated chloride channel. The protein consists of two transmembrane domains, which contribute to the ion pore with two cytoplasmic nucleotide-binding domains (NBDs) linked by a cytosolic regulatory domain. Chloride transport is controlled by cAMP-dependent protein kinase A (PKA) phosphorylation of the R-domain with ATP binding and hydrolysis at the NBDs.76,77

CFTR is expressed on the apical plasma membrane of the epithelial cell, where it is part of a multiprotein assembly in close proximity to a number of other ion channels and membrane receptors.78 In addition to acting as a gated-chloride channel, CFTR appears to play a role in the regulation of other apical ion transport processes, including the epithelial sodium channel (ENaC) whose activity is inhibited by CFTR in the normal airway.78,79 In addition to CFTR, luminal chloride secretion in airway epithelial cells can also occur through alternative chloride channels such as those activated by P2Y2 receptors or intra-
cellular calcium. These fluid and ion secretion and absorption processes are responsible for maintaining appropriate airway surface liquid (ASL) hydration.

In the airways, the most important factor in mucus clearance is hydration of the airway surface. The primary physiologic defect in the airways in CF is a thick, tenacious mucus that is poorly cleared due to dehydration of the ASL. The ASL consists of fluid in the mucus layer and the periciliary liquid (PCL). The mucus layer of the airway lies over the PCL, which is a low viscosity polyanionic fluid or gel layer that facilitates ciliary movement.\(^{75}\) Thus, the PCL serves as a lubricant between the mucus layer and the airway allowing ciliary movement of the mucus layer. ASL volume regulation is not completely understood; however, in the normal lung it appears that chloride secretion by the CFTR and alternative chloride channels, and sodium absorption via ENaC, work in conjunction to maintain ASL. The balance between sodium absorption and chloride secretion maintains the proper hydration and tonicity of secretions. In CF, the CFTR is either missing or nonfunctional; thus, this balance is disrupted. In addition to the loss of CFTR chloride secretion, ENaC-related sodium absorption appears to be increased leading to dehydration of the ASL, collapse of the PCL, and the inability of airway cilia to clear mucus.\(^{75,79}\) Epithelial cells in CF retain their ability to secrete chloride via non-CFTR chloride channels; however, this function is not sufficient to maintain ASL homeostasis.

The dehydration of the mucus and the loss of the PCL results in adhesion of mucus to the airway surface and the formation of mucus plaques. Adhesive secretions obstruct submucosal glands and the distal airways.\(^{77}\) Mucus stasis and airway obstruction occur. As a result of mucus stasis, inhaled bacteria are not efficiently cleared, and bacterial colonization develops resulting in a neutrophilic inflammation. Mucus stasis may prevent neutrophil migration and the diffusion of antimicrobial substances produced in the lungs, decreasing the efficiency of the immune reaction. In addition, neutrophil elastase and other related proteases damage structural proteins in the lungs leading to bronchiectasis and decreased opsonophagocytosis, which perpetuates chronic infection.\(^{80}\) Furthermore, deoxyribonucleic acid (DNA) and other breakdown products released upon neutrophil death contribute to decreased mucus viscoelasticity. Thick, viscous mucus may also create a hypoxic environment that is favorable for bacterial growth and biofilm development. In addition, evidence exists demonstrating impaired production or trafficking of CFTR may cause activation of nuclear factor-kappa B resulting in increased interleukin-8, the principal neutrophil chemoattractant in the lung; therefore, airway inflammation may also occur independent of infection in the CF lung.\(^{80,81}\)

The genetic defect in CF results in episodic exacerbations of acute viral and bacterial pulmonary infections, leading to further airway structural damage, bronchiectasis, air trapping, and hyperinflation of the lungs. Increased airway obstruction and a progressive decline in lung function in turn lead to chronic hypoxia.

**Genetic Defects**

The expression and activation of CFTR is complex. Briefly, messenger ribonucleic acid (mRNA) transcribed from DNA in the nucleus migrates to cytoplasmic ribosomes where amino acid translation occurs. Protein synthesis then occurs at the membrane of the endoplasmic reticulum followed by glycosylation and folding of the protein in the Golgi body. In normal cells, appropriately folded CFTR is then trafficked to the cell surface. Truncated, unstable, or misfolded protein is degraded by the endoplasmic reticulum.\(^{82}\)
Mutations of the CFTR can be classified based on how they disrupt CFTR function (Table 11-5). Class I mutations are splicing or nonsense mutations and are caused by a premature stop codon, which results in an unstable mRNA or a shortened or unstable protein that is degraded in the cell. Class I mutations result in the CFTR not being expressed at the cell membrane. Molecules that suppress premature stop codons and allow translation to continue may be used to increase apical membrane protein expression in this class of mutations. This type of mutation is associated with 5–10% of CFTR mutations and results in a severe phenotype exhibiting both pulmonary disease and pancreatic insufficiency. The W1282X mutation is the most common mutation among CF patients of Ashkenazi Jewish descent where it is seen in up to 60% of alleles; however, it is rare in other populations.

Class II mutations are caused by impaired processing of the CFTR protein, which results in degradation of CFTR by the endoplasmic reticulum degradation processes, and a lack of functional CFTR expression at the cell membrane. Class II mutations are similar to Class I mutations; however, they result in a more severe form of CF that includes respiratory disease and pancreatic insufficiency. The most common Class II mutation, ∆F508, is caused by the deletion of a phenylalanine at the 508 amino acid position. This mutation is seen in 70% of defective alleles and 90% of CF patients in the United States. Because of its frequency, most research has been directed at understanding and correcting this defect. It is thought that calnexin and the heat shock protein HSC70 are involved in the trafficking of the protein as it matures. They may associate with the protein and assist it in the folding process before releasing it to the Golgi body for glycosylation. Because the ∆F508 mutation impedes proper folding, a prolonged association with calnexin and HSC70 results in ubiquitination and degradation in the endoplasmic reticulum. It is estimated that greater than 99% of ∆F508 protein is degraded by this mechanism. When expressed at the membrane, the ∆F508 mutation retains chloride-channel activity, but it does not

Table 11-5

<table>
<thead>
<tr>
<th>Functional defect</th>
<th>Class I</th>
<th>Class II</th>
<th>Class III</th>
<th>Class IV</th>
<th>Class V</th>
<th>Class VI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Premature stop codon</td>
<td>Amino acid deletion</td>
<td>Amino acid substitution</td>
<td>Amino acid substitution</td>
<td>Promoter or splicing errors</td>
<td>Truncated at C-terminus</td>
<td></td>
</tr>
<tr>
<td>Shortened dysfunctional protein that is degraded in ER</td>
<td>Misfolding of protein that is degraded in ER</td>
<td>Dysregulation of protein at cellular membrane</td>
<td>Decreased channel architecture leading to decreased conductance or channel gating</td>
<td>Unstable at plasma membrane</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Representative genotypes</th>
<th>Class I</th>
<th>Class II</th>
<th>Class III</th>
<th>Class IV</th>
<th>Class V</th>
<th>Class VI</th>
</tr>
</thead>
</table>

CFTR, cystic fibrosis transmembrane conductance regulator; ER, endoplasmic reticulum.
function as well as the wild-type channel. This decrease in relative function may be related to a decreased open time for the channel.

Class III mutations are caused by full-length proteins that are properly processed and trafficked to the membrane but have significantly decreased chloride ion transport capabilities. The lack of channel activity appears to be caused by the protein’s resistance to phosphorylation, ATP binding, or hydrolysis. The most common Class III mutation is a glycine to aspartic acid interchange at amino acid position 551 (G551D), which is present among 3% of CF chromosomes. Patients with Class III mutations usually present with severe disease that includes both pancreatic insufficiency and respiratory disease.

Class IV mutations, like Class III mutations, are properly processed and expressed at the apical membrane. The protein is appropriately regulated by PKA and cAMP; however, amino acid substitutions result in changes in channel architecture that alter chloride conductance or channel gating. These mutations are relatively rare. When expressed on the apical membrane, the ΔF508 mutant CFTR retains some chloride conductance and may exhibit Class IV mutant properties. Because the CFTR protein is appropriately expressed on the apical membrane and maintains some function, the mutation is associated with pancreatic sufficiency and milder disease.

Class V mutations result from promoter or splicing errors, leading to decreased expression of the functional protein at the apical membrane. This may be due to decreased numbers of the CFTR protein or adequate numbers with decreased function. Approximately 13% of CFTR mutations result in pre-mRNA splicing errors. Presentation of CF in patients with Class V mutations is quite variable, and disease severity has been shown to be inversely related to the number of correctly spliced transcripts. Mutations that generate both correctly and aberrantly spliced transcripts (3849 + 10kb C→T) confer a milder phenotype with more variable disease, whereas mutations that completely abolish exon recognition (621 + 1 G→T) result in an absence of correctly spliced transcripts and a relatively severe phenotype. Additional classification categories have been used to describe mutations that result in increased protein turnover at the membrane or proteins presenting with altered regulatory properties.

Class VI mutations result from expression of a truncated CFTR protein that lacks 70-98 residues at the C-terminus of CFTR. An example of this is Q1412X, which lacks 70 amino acids at the C-terminus and has reduced stability at the apical membrane surface.

**CLINICAL PEARL**

Although the most common mutation in Caucasians in the United States is the ΔF508 mutation, the prevalence of mutation in other races and ethnicities is not as predictable. Population mixing may alter the prevalence of mutation in different racial and ethnic groups over time.

**Treatment**

Current treatment options available to help manage the symptoms of CF are outlined in Table 11-6. Management of CF symptoms consists of replacing pancreatic enzymes, correcting nutritional deficiencies, and preventing pulmonary deterioration through averting and treating infectious exacerbations. These measures are supportive/palliative and
help in managing the clinical manifestations of CF. There has been a strong interest in the development of drugs that can restore CFTR activity and significantly alter the course of CF. Because CF is caused by a variety of different mutations, different drugs will be necessary for treating it based on the underlying genetic defect. The two main classes of drugs that are being developed for modulating CFTR activity are **potentiators** and **correctors**. Molecules aimed at improving the function of the CFTR protein by increasing chloride conductance are termed “potentiators.” Agents aimed at improving the expression of CFTR on the apical membrane through enhanced gene transcription, protein processing, or trafficking of the protein are termed “correctors.”

Two disease-modifying drugs that correct the underlying defect in CF have been recently approved for the treatment of CF. The approval of these drugs heralds a new era in the treatment of CF and exemplify how new drugs are being developed to target specific mutations.

### Table 11-6

<table>
<thead>
<tr>
<th>Therapy</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microencapsulated pancreatic enzymes</td>
<td>Pancreatic insufficiency leads to malnutrition and fat-soluble vitamin deficiency due to decreased digestion and absorption of dietary fat and protein and fat-soluble vitamins.</td>
</tr>
<tr>
<td>Multivitamin</td>
<td>It is used for supplementation of fat-soluble vitamins. Percussion and postural drainage It increases clearance of airway mucus.</td>
</tr>
<tr>
<td>Recombinant human DNase</td>
<td>It decreases viscosity of airway mucus, increases clearance of airway mucus, improves pulmonary function, and decreases frequency of pulmonary exacerbations.</td>
</tr>
<tr>
<td>Hypertonic saline (7%)</td>
<td>It may improve hydration of airway surface liquid, improves lung function and quality of life, and decreases exacerbations.</td>
</tr>
<tr>
<td>β-agonists/theophylline</td>
<td>It improves pulmonary function in patients with reactive airway disease. Responsiveness to bronchodilators should be identified prior to initiating chronic therapy.</td>
</tr>
<tr>
<td>Corticosteroids</td>
<td>Oral corticosteroids (1–2 mg/kg) decrease inflammation associated with disease but have undesirable effects. The efficacy of inhaled corticosteroids is not proven.</td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>High-dose ibuprofen decreases CF-associated inflammation; however, there is an increased risk of gastrointestinal adverse effects.</td>
</tr>
<tr>
<td>Aerosolized tobramycin</td>
<td>It improves lung function and quality of life. It decreases pulmonary exacerbations and intravenous antibiotic use.</td>
</tr>
<tr>
<td>Azithromycin</td>
<td>It is recommended for use in patients 6 years of age or older with <em>Pseudomonas aeruginosa</em>. Treatment improves lung function.</td>
</tr>
</tbody>
</table>

CF, cystic fibrosis.
**Ivacaftor (Kalydeco)**

Ivacaftor (VX-770) is an orally administered, small molecule drug that acts as a potentiator and enhances the gating activity of CFTR channel. It was identified via high-throughput screening from a library consisting of over 230,000 compounds.\(^{92}\) Ivacaftor was originally evaluated for treatment in patients who carried a G551D mutation in at least one allele. G551D mutations result in a glycine to aspartic acid change that allows localization of CFTR to the cell surface but diminishes their gating ability. In two 48-week, placebo-controlled, phase III trials in patients aged 12 and older (STRIVE) or 6-11 years (ENVISION), 150 mg ivacaftor every 12 hours resulted in a significant improvement in lung function as measured by FEV\(_1\).\(^{93,94}\) Safety and efficacy of ivacaftor were monitored in patients who completed the STRIVE and ENVISION trials for an additional 96 weeks in a phase III, open-label extension study (PERSIST).\(^{95}\) The improvements in FEV\(_1\) were maintained, and the drug was found to be generally well tolerated at nearly three years of treatment. Ivacaftor received approval from the FDA in 2012 for the treatment of patients who tested positive for a G551D-CFTR variant in at least one allele. In 2014, the FDA extended the approval to eight additional Class III mutations (G1244E, G1349D, G178R, G551S, S1251N, S1255P, S549N, S549R) and for a Class IV mutation (R117H).\(^{96}\)

Because ivacaftor acts as a potentiator, it is only effective when malfunctioning CFTR is expressed on the cell surface. Thus, ivacaftor alone is unlikely to be effective for treating patients suffering from Class I and Class II mutations such as \(\Delta F508\) mutation and G542X, which affect the expression or localization of CFTR to the cell surface. Based on the mechanism of action and available clinical data, ivacaftor is not currently indicated for patients with CF due to mutations other than the selected Class III and Class IV mutations listed above.

**Lumacaftor/Ivacaftor Combination (Orkambi)**

The approval of ivacaftor marked a significant milestone in the treatment of CF, as it was the first drug to be approved that treated the underlying defect in CF. However, ivacaftor alone is not effective for treating individuals who are homozygous for the \(\Delta F508\) mutation. \(\Delta F508\) is the most common cause of CFTR mutation, and approximately 45% of the CF patients in North America are homozygous for the \(\Delta F508\) mutation.\(^{97}\) A combination of a “potentiator” such as ivacaftor with any other “corrector” molecule is likely going to be the most effective treatment for patients homozygous for the \(\Delta F508\) mutation. The “corrector” can improve CFTR folding and trafficking, while the “potentiator” can help increase the probability of channel opening.\(^{97,98}\) Lumacaftor (VX-809) is a corrector that has been demonstrated to improve folding and trafficking of \(\Delta F508\) CFTR to the cell surface. In addition, it has been shown to help improve stabilization of the partially rescued \(\Delta F508\) CFTR at the cell surface by binding to the protein.\(^{99}\)

A phase 2 study in patients homozygous for the \(\Delta F508\) mutation showed that a combination of lumacaftor and ivacaftor resulted in improvements in FEV\(_1\), and also modest improvement in sweat chloride concentration.\(^{100}\) Two phase 3 randomized, double-blind, placebo-controlled trials (TRAFFIC and TRANSPORT) were conducted to test the efficacy and safety of ivacaftor with two different doses of lumacaftor. Patients were randomly assigned to one of the following three groups: 600 mg lumacaftor once daily with 250 mg ivacaftor every 12 hours, 400 mg lumacaftor and 250 mg ivacaftor every 12 hours, or lumacaftor and ivacaftor matched placebos every 12 hours. A total of 1,122 patients were randomized (559 in TRAFFIC and 563 in the TRANSPORT study), and the regimen was for
24 weeks. A significant improvement in FEV₁ and reductions in rate of pulmonary exacer-
inations at both lumacaftor doses, for patients receiving the lumacaftor–ivacaftor combi-
nation, was seen by day 15 and continued through the 24-week regimen. Adverse effects
reported by the treatment and placebo groups were comparable and suggested that the
drug combination has an acceptable side-effect profile.⁹⁷ Orkambi (lumacaftor/ivacaftor)
was approved on July 2, 2015 for the treatment of CF patients 12 years of age and older
who have two copies of the ΔF508 mutation in the CFTR gene.¹⁰¹

CLINICAL PEARL

Kalydeco (lumacaftor) is indicated for CF caused by the G551D, G1244E, G1349D, G178R, G551S, S1251N, S1255P, S549N, S549R or the R117H
mutations. Orkambi (lumacaftor/ivacaftor) is indicated for patients with two
copies of ΔF508 mutation in their CFTR gene.

Other Protein Modulators in Development

Other compounds are being investigated for their ability to correct the underlying defect in
CF by targeting the processing, trafficking, and activity of CFTR.¹⁰²

GENTAMICIN. Gentamicin increases CFTR expression in CFTR nonsense mutation trans-
ferred cells and in a bronchial epithelial cell line expressing a nonsense mutation.¹⁰³,¹⁰⁴
Gentamicin nasal drops (given 3 times a day for 14 days) caused a significant decrease in
nasal potential difference (NPD), a measurement of chloride secretion and sodium absorp-
tion across the epithelia, in response to a chloride-free isoproterenol solution in patients
homozygous for the W1282X stop mutation (n = 4) or heterozygous for the W1282X and
G542X (n = 3), ΔF508 (n = 1), or 3849 + 10kb C→T (n = 1) mutation.¹⁰⁵ No differences were
seen in basal NPD.

In a followup double-blind, placebo-controlled crossover trial, nasally administered
gentamicin was evaluated in 19 CF patients homozygous for the W1282X mutation (n = 11) or
heterozygous for the W1282X and ΔF508 mutations (n = 8) and five patients homozygous for
the ΔF508 mutation.¹⁰⁶ In patients carrying the W1282X mutation (homozygous or hetero-
zygous), a significant decrease in basal and isoproterenol–treated NPD was associated with
gentamicin administration; however, the response was not seen in all patients. When homo-
yzogous and heterozygous W1282X patients were evaluated separately, NPD was significantly
decreased in the homozygous population but not the heterozygous population. No changes
in basal NPD or response to chloride-free isoproterenol solution were seen in patients
homozygous for ΔF508. Ex vivo analysis of full-length CFTR protein in nasal epithelial cells
before and after gentamicin treatment in two heterozygous patients who had a response to
therapy demonstrated an increased cellular membrane localization of CFTR.

Another study evaluating intranasal gentamicin and tobramycin in CF patients hetero-
yzogous for a premature stop mutation (n = 11) or CF patients without a stop mutation
(n = 18) found no differences in basal or isoproterenol stimulated NPD over 28 days.¹⁰⁷
Ex vivo evaluation of nasal epithelial cells likewise demonstrated no increases in membrane
localization of CFTR. The effect of systemically administered gentamicin was evaluated in
five CF subjects heterozygous for one premature stop mutation and five CF subjects with-
out premature stop mutations.¹⁰⁸ Subjects were administered gentamicin for 7 days with
doses adjusted to achieve peak serum concentrations of 8–10 mg/L and trough concentrations <2 mg/L. Chloride sweat test, basal NPD, and response to chloride-free isoproterenol administration were measured at baseline, during (days 3, 4, 5, and 6) and after gentamicin administration, and again 1 to 4 weeks after treatment. No difference between the two groups was seen in the chloride sweat test or basal NPD; however, patients with the stop mutation had a significant increase in the number of NPD readings, indicating increased chloride transport. As with the other studies, not all patients responded.

Differences in these trials may be related to population differences because it has been demonstrated that response to gentamicin may be related to initial levels of CFTR expression, enhanced nonsense-mediated mRNA decay, or to the type of stop mutation. Furthermore, early studies demonstrating success with gentamicin have been conducted in populations of patients having at least one mutation containing W1282X. It has been demonstrated that W1282X CFTR retains partial chloride channel function that is enhanced after suppression of the stop codon. Finally, although none of the trials reported significant adverse effects, long-term systemic administration of gentamicin would raise safety concerns because of its well-documented toxicities.

**ATALUREN.** Because of the early success with gentamicin and the recognized toxicity and administration issues, considerable interest exists in the development of compounds that can safely suppress premature stop codons. Ataluren is an orally available oxadiazole identified via high throughput screening of low molecular weight compounds that may have the potential to treat disease caused by nonsense mutations. At low concentrations, ataluren has been shown to promote dose-dependent read through in nonsense mutations and is a more potent nonsense suppressor than gentamicin.

Phase I trials of ataluren indicated the drug was safe for further clinical study. A phase II trial in adult CF patients with at least one nonsense mutation in the CFTR gene evaluated the effectiveness of ataluren given in two 28-day cycles. The first cycle (n = 23) consisted of 16 mg/kg per day of ataluren given in 3 divided doses for 14 days followed by 14 days without drug. In cycle two, 21 of the cycle one patients received ataluren 40 mg/kg/day in 3 divided doses for 14 days followed by 14 drug-free days. Significant increases were seen in total chloride transport in both treatment cycles, and total chloride transport entered the normal range for 57% of patients in cycle 1 and 43% of the patients in cycle 2. Adverse effects were mild. Efficacy and safety were also studied for patients receiving ataluren 3 times daily for 12 weeks. A positive improvement in pulmonary function was observed over time in this study. A randomized, placebo-controlled phase III trial to assess safety and efficacy of ataluren showed that the side effects were similar between the placebo and treatment groups. However, no significant improvement in lung function was observed in the general nonsense mutation CF population. Only patients not receiving chronic inhaled tobramycin seemed to show an improvement in lung function with ataluren. Based on these studies, ataluren seems to be well tolerated and may be useful for nonsense mutation CF patients who are not receiving chronic inhaled tobramycin.

**SODIUM BUTYRATE.** Class V mutations are caused by aberrantly spliced transcripts. The severity of the disease depends on the number of correctly spliced transcripts. One method for increasing the numbers of correctly spliced transcripts, and thus the expression of CFTR at the apical membrane, is to overexpress splicing factors which increases the amount of correctly spliced RNA. Sodium butyrate has been previously shown to modify the alternative splicing pattern of exon 7 in the survival motor neuron-2 (SMN2) gene, thus increasing
the number of full-length SMN mRNA transcripts and exon 7 containing SMN protein in spinal muscular atrophy lymphoid cells. Based on these results, the effect of sodium butyrate function and expression of CFTR in an epithelial cell line from a nasal polyp of a patient with the 3849 + 10kb C→T mutation was evaluated. Sodium butyrate significantly decreased the amount of aberrantly spliced transcripts and activated CFTR. The utility of these findings is unknown as sodium butyrate is not well suited for clinical use; however, other agents such as valproic acid have also demonstrated the ability to increase SMN transcripts and proteins and may have application in this area.

**SODIUM 4-PHENYLBUTYRATE.** Sodium 4-phenylbutyrate (4-PBA) is a derivative of sodium butyrate that has been shown to increase trafficking of ΔF508. It is thought that 4-PBA reduces the expression of heat shock protein HSC-70, resulting in decreased HSC-70 association with ΔF508 leading to decreased ΔF508 degradation in the endoplasmic reticulum. The effect of 4-PBA on NPD and sweat chloride response was evaluated in CF patients homozygous for the ΔF508 mutation in a randomized, double-blind, placebo-controlled trial. Subjects were given placebo or 19 grams of 4-PBA in divided doses 3 times a day. Patients in the pilot trial treated with 4-PBA (n = 9) showed significant improvement in NPD after infusion of a chloride-free isoproterenol solution when compared to placebo-treated patients. No differences were seen in amiloride sensitive NPD or sweat chloride concentrations.

A phase I/II trial of 4-PBA in 19 adult CF patients homozygous for ΔF508-CFTR demonstrated a significant increase in isoproterenol-stimulated chloride transport in nasal epithelia. The maximal response was seen with a daily dose of 20 grams for 1 week. Peak response occurred between days 3 and 4 with a decrease in response seen at day 7. It is possible that this decrease in response could be related to increased ENaC activity because a recent in vitro study showed that 4-PBA induces a time-dependent increase in ENaC protein in the apical membrane in conjunction with increased apical amiloride-sensitive sodium current. Therefore, increased CFTR expression may be offset by increased ENaC-related sodium absorption.

**GENISTEIN.** Genistein acts as a potentiator and has been investigated for its ability to increase CFTR channel opening and treat the deficient channel function caused by Class III mutations. Genistein, a soybean-derived isoflavone, has been shown to increase CFTR chloride transport in both wild-type and mutant CFTR. In patients with at least one G551D mutation, perfusion of the nasal mucosa with genistein was shown to hyperpolarize NPD in both healthy and CF patients indicating it stimulates chloride conductance in the nasal epithelia. It appears that genistein enhances CFTR activity by decreasing the closing rate of the channel at low concentrations, but it may inhibit channel activity at high doses by binding to a low affinity site that decreases the opening rate of the channel. Genistein’s clinical usefulness may be limited by its low potency and rapid metabolism.

**PDE5 INHIBITORS.** PDE5 inhibitors have been shown to correct ΔF508-CFTR trafficking and CFTR chloride conductance. Intraperitoneal injections of sildenafil, vardenafil, and tadalafil have been shown to increase CFTR-mediated chloride transport in ΔF508del mice. Vardenafil was also found to stimulate chloride conductance through the normal CFTR protein. These promising early results suggest that PDE5 inhibitors could be useful for rescuing ΔF508-CFTR trafficking and restoring deficient chloride conductance caused by Type II mutations.
Additional disease-modifying agents that are in development include Riociguat, QBW251, N91115 and QR-010. The progress of these agents through the discovery and regulatory process can be monitored at the CF Foundation website (http://www.cff.org/research/drugdevelopmentpipeline/).

Gene Therapy

Because defective genes are responsible for CF, introduction of a normal copy of the CFTR gene can help to correct the underlying defect in CF. Adeno-associated viruses (AAV) have been tested for their ability to deliver the complete human CFTR cDNA to the lungs, sinus, and nose of individuals with CF. Aerosolized AAV vectors encoding CFTR were found to be safe in a study involving 102 subjects. However, no significant improvement in lung function was observed, most likely owing to the challenges associated with delivering genes to the lungs. More recently, a randomized, double-blind, placebo-controlled, phase 2b trial conducted in the UK found a modest but significant improvement in lung function following delivery of plasmid DNA encoding the CFTR gene using liposomes. The results reported in this study are very encouraging, but several challenges will need to be overcome before gene therapy finds a place in the treatment of CF.

SUMMARY

Pharmacogenetic testing can help guide drug therapy for both asthma and CF. Genetic variants have been identified that influence the response to treatment with $\beta_2$-agonists, inhaled corticosteroids, LT modifiers, and theophylline in asthmatic patients. The application of this research can help practitioners to optimize therapy based on genotype and reduce negative outcomes. Mutations in the CFTR gene are responsible for CF. The approval of ivacaftor and lumacaftor/ivacaftor for specific mutations has ushered in a new era in the treatment of CF. Although each of these drugs treats the underlying defect in CF and improves CFTR function, they are indicated for different mutations and demonstrate how personalized medicine is revolutionizing the way diseases are treated.

REFERENCES


