Pharmacogenetics and Pharmacogenomics of Airway Diseases

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I. Introduction
Asthma and chronic obstructive pulmonary disease (COPD) are complex syndromes of airway inflammation. Asthma is a disease of the small airways of the lung. Intermittent narrowing of the respiratory bronchioles produces airway limitation and the symptoms of wheezing, chest tightness, and breathlessness. By contrast, in COPD the limitation of airflow is poorly reversible and usually gets progressively worse over time. The disease is primarily, but not exclusively, seen in smokers and former smokers. Environmental and genetic factors contribute to the etiology of both diseases. Cigarette smoking is the main risk factor for COPD, although less than 20% of chronic heavy smokers will develop symptoms of airway obstruction (1). Bronchodilators and corticosteroids are currently the most common medications used in the treatment of asthma and COPD.

The term “pharmacogenetics” was originally used to describe clinical observations of inherited differences in drug effects in 1950s (2). It is now defined as the study of interindividual variations in DNA sequence (polymorphisms) related to drug response (3,4). It is the ambition of pharmacogenetic studies that the analysis of variation of a specific gene or group of genes may be used to predict responses to a specific drug or class of drugs. Genetic variation in a population is detected by assays of individual genotypes. Traits such as drug responses may be determined by environmental as well as genetic influences. Genetic variation also underlies the differential susceptibility of organisms to diseases and sensitivity to toxins.

Most drug effects are determined by the interplay of several gene products that influence the pharmacokinetics and pharmacodynamics of medications, including inherited differences in drug targets (e.g., receptors), drug disposition (e.g., metabolizing enzymes and transporters) (5), drug metabolism (3), and drug adverse reaction (6). It is estimated that genetic polymorphisms can account for 20% to 95% of variability in drug disposition and effects (5).

Although the terms pharmacogenetics and pharmacogenomics are synonymous for all practical purposes, pharmacogenomics uses genome-wide approaches to study all the genes that influence drug responses (5) while pharmacogenetics involves the study of a single gene’s interactions with drugs. The pharmacogenomics approach tends to be applied to identify genes in the search for novel drug targets. This is in contrast to traditional drug design that depends on a prior knowledge of the target and is based on high-throughput screening of such targets to identify small-molecule antagonists or agonists.
The successful completion of the Human Genome Project was regarded as one of the greatest scientific achievements in the last 50 years. The project identified thousands of protein-coding genes in the human genome, many of which could be important targets for drug development. On the basis of the project, a second generation human haplotype map of over 3.1 million single nucleotide polymorphisms (SNPs) was finished in 2007 (7). The public databases of these findings together with genome sequence of other species such as the mouse provide extremely useful resources for pharmacogenetics and pharmacogenomics of clinical practice.

In this chapter, we review how pharmacogenetic and pharmacogenomic approaches have developed in the search for novel drug treatments for airway disease. In addition, we summarize the current progress for investigating genetic variations to common drugs that are used in asthma and COPD.

II. Approaches for Studying Pharmacogenetics and Pharmacogenomics

The human genome has about 20,000 to 25,000 genes (8). The identification of all human genes and their regulatory regions provides the essential framework for understanding the molecular basis of diseases. It also provides a foundation for the future of pharmacogenomics studies. Rapid developments in global gene analysis, gene product analysis, siRNA, targeted molecular genetic testing, and medical bioinformatics is already changing the practice of medicine (9). The study of genetic variation has the potential to understand individual variation in mechanisms of airway disease and the response of patients to treatment. However, no single variant interrogated in airway diseases has yet been found to explain more than 10% of the total phenotypic variance of treatment response of any drug (10).

The forced expiratory volume in one second (FEV1) and peak expiratory flow (PEF) are the measures of airflow usually studied for drug response in airways disease. These parameters can be used to measure the response to inhaled bronchodilators, corticosteroids, and other treatments. Other traits that are worthy of study by pharmacogenetics include drug metabolism [e.g., clearance of theophylline (TP) in serum] and drug adverse effects. Since the mid-1990s genes involved in most airway drug targets have been successfully screened. The polymorphisms within these genes have been identified, and the associations between these polymorphisms and airway drug’s response have been intensively investigated.

A. Human Genome Variation and Pharmacogenetics Research

Common genetic variants are observed between humans at both the individual and the population levels. SNPs are the most frequent variants found in the genome, accounting for 90% of human genetic variation. Recent whole genome sequencing has revealed approximately 10 million SNPs in the human genome (11,12). SNPs have been found within coding sequences of genes (exons), noncoding regions of genes (introns), as well as within intergenic regions. Examples of exonic SNPs are those found within the β2-adrenergic receptor gene (ADRB2) that have been linked to altered response to β2-agonists.

Insertion and deletion of short segments of DNA (INDEL) is another type of common polymorphism. More than 500,000 INDELs are distributed throughout the
human genome, with approximately 36% of them being located within promoters, introns, and exons of known genes (11). INDELs can have a significant impact on gene function not only when present in exonic coding sequence but also when within a gene intron. For example, an INDEL polymorphism of a 287 bp Alu sequence in intron 16 of the angiotensin-I converting enzyme accounts for 50% of the variance of serum enzyme levels. Homozygosity for the insertion allele (II genotype) results in individuals having significantly lower serum enzyme levels than carriers of the deletion allele (ID and DD genotypes) (13).

VNTRs (variable number of tandem repeats) polymorphisms are widespread in the genome and contain variable numbers of repeated nucleotide sequences that result in alleles of varying lengths. Because of their large numbers of alleles, VNTR loci therefore typically have high levels of heterozygosity that make them very informative for genetics research. A VNTR mutation in the promoter of the arachidonate 5-lipoxygenase (ALOX5) gene has been shown to have an effect on the efficiency of both inhibitors and antagonists of its receptor (14).

Inversions may involve larger regions of the genome in which a segment of a chromosome is reversed end to end and occur when a chromosome breaks in two places. Interestingly, a 900-kb inversion has been identified within a large region of conserved linkage disequilibrium (LD) on human chromosome 17, and contained within the inversion region is the corticotrophin-releasing hormone receptor 1 gene (CRHR1). Polymorphisms within CRHR1 have been shown to be associated with inhaled corticosteroid (ICS) response in asthma (15).

A copy number variant (CNV) is a segment of DNA for which there are more or less than two copies in the genome. The genetic segment involved may range from one kilobase to several megabases in size (16). Copy number variation is not only about quantity but also about quality. A variety of techniques can allow the detection and discovery of CNVs including cytogenetic techniques such as fluorescent in situ hybridization, comparative genomic hybridization, array comparative genomic hybridization, and by large-scale SNP genotyping.

A further type of variation involves RNA rather than DNA. RNA alternative splicing occurs when the exons of a primary gene transcript, the pre-mRNA, are separated and reconnected so as to produce alternative ribonucleotide arrangements. These linear combinations then undergo the process of translation where unique sequences of amino acids are specified, resulting in different protein isoforms. Alternative splicing includes intron retaining mode, exon cassette mode, and alternative donor/acceptor site mode.

B. Methods for Identification of Gene Variation Underlying Drug Responses

There are a variety of approaches that can be applied to identify potential genes underlying drug responses. These include candidate genes analysis and positional cloning, genome-wide association (GWA) studies, gene expression profiling, and microRNA targeting.

The candidate gene approach examines particular genes known to have specific roles in targeting, metabolism, and disposition of drugs. Variations in these genes can be compared between cases exhibiting particular drug responses and appropriate controls. An excellent example of this type of study in the case of airway diseases is the
examination of mutations of the glucocorticoid receptor (GR) gene and their effect on
the response to corticosteroid therapy.

Positional cloning involves the detection of genetic linkage between chromosome
region and drug response traits through the study of families. Subsequent fine mapping
of the genetically linked region leads eventually to identification of the gene underlying
drug responses. The vast number of SNPs identified throughout the genome and
developments in microarray technology mean that positional cloning has now been
superseded by GWA studies.

GWA studies involve genotyping thousands of samples, either as case control
cohorts or in family trios, for hundreds of thousands of SNPs distributed throughout the
genome. Genotyping is followed by the comparison of the frequencies of either single
SNP alleles, genotypes, or multimarker haplotypes between drug response traits and
controls. GWA studies allow the identification of trait susceptibility genes with only
modest increases in risk. A recent example of a GWA in pharmacogenetics was the
study of myopathy in myocardial infarction patients receiving simvastin treatment. A
GWA scan of only 90 patents with myopathy and 90 controls identified a single strongly
associated SNP, rs4363657. This initial result led on to the identification of a common
mutation in SLCOB1B1 that was strongly associated with an increased risk of statin-
induced myopathy. It is likely that genotyping of the SLCOB1B1 variants will help to
achieve the benefits of statin therapy more safely and effectively (17).

Gene expression profiling is the measurement of the expression (transcript
abundance) of thousands of genes simultaneously and (ideally) genome wide, to create a
global picture of cellular function. These gene expression profiles can, for example,
distinguish between cells that are actively dividing, or show how cells react to a par-
ticular drug treatment. The data generated from such genome-wide expression experi-
ments provide enormous information about the genetic pathways involved in regulation
of cell cycle, RNA processing, DNA repair, immune responses, and apoptosis (18).

The discovery of small “noncoding” or “nonmessenger” RNA molecules that are
repressors of translation (microRNAs) has provided the opportunity to specifically
suppress a gene or clusters of genes. The recent employment of synthetic analogs of
these small RNA molecules termed “antagomirs” has shown that microRNAs of interest
can be specifically targeted. Understanding the role of microRNAs in fundamental
processes associated with complex diseases has the potential of being of assistance in
disease diagnosis, prognosis, and may also result in the identification of new therapeutic
targets (19).

Many, if not all, of the above techniques rely heavily on bioinformatics, that is,
the application of information technology to the field of molecular biology. Bio-
informatics entails the creation and advancement of databases, algorithms, computa-
tional and statistical techniques, and theory to solve formal and practical problems
arising from the management and analysis of genomic data. The size of the task is
exemplified by the completion of whole genome sequencing for more than 40 species.
Major research efforts in bioinformatics include sequence alignment, gene finding,
genome assembly, protein structure alignment, protein structure prediction, prediction of
gene expression and protein-protein interactions, and the modeling of evolution.

For airway diseases, application of bioinformatics to expression quantitative trait
loci (eQTL) has the potential for discovery of novel loci that contribute to heterogeneity
in response to asthma and COPD pharmacotherapy. Additionally, the development of
statistical models that predict the genomics of response to airway drugs will complement SNP discovery in moving toward personalized medicine.

C. Molecular Tools for Pharmacogenetics

When polymorphisms of genes that have suspected pharmacogenetic effects have been identified, it is possible to study their functional roles by applying molecular biologic approaches. Short interfering/silencing RNA (siRNA), transfection, electrophoretic mobility shift assay (EMSA), and mouse models are at present the most commonly utilized methods to try and establish the functional consequences of gene variants.

siRNA is a class of 20 to 25 nucleotide-long double-stranded RNA molecules that play a variety of roles in biology. Most notably, siRNA is involved in the RNA interference (RNAi) pathway, where it alters the expression of specific genes. Although just one decade has passed since the discovery of RNAi, human clinical trials of RNAi therapy are currently occurring for diseases such as macular degeneration, respiratory syncytial virus infection, hepatitis, pachyonychia congenita, and solid tumors (20).

Transfection is a technique that allows one to study gene function by artificially introducing the foreign DNA into cultured eukaryotic cells. Stable integration of the DNA into the recipient genome and subsequent examination of both RNA and protein expression of the gene allows ascertainment of the pharmacogenomic effect of gene polymorphisms. An EMSA, also referred to as a gel shift assay, band shift assay, or gel retardation assay, is a common technique used to study protein-DNA or protein-RNA interactions. It is particularly useful to study the polymorphisms within regulatory sequences and introns of a gene. This procedure can determine if a protein or mixture of proteins is capable of binding to a given DNA or RNA sequence thereby effecting its expression, and can sometimes indicate if more than one protein molecule is involved in the binding complex.

The most important issue in pharmacogenetics is the testing of identified functional polymorphisms in the range of populations who may receive the drug. Retrospective, prospective studies, and finally, the study of random samples by way of clinical trials are all required to validate potential pharmacogenetic loci. Only then can personalization of medication in clinical care be feasible (Fig. 1).

Difficulties in obtaining direct access to human tissues means that direct determination of the mechanism of action of polymorphisms may be almost impossible. Mouse models can, however, bridge the gap between in vitro cell-based studies and human clinical traits. They provide a unique platform for investigation of altered drug response as well as being a tool for potential drug discovery (21). A large degree of syntenic homology exists between the mouse and the human genomes. Nearly all human genes have counterparts in the mouse, which can usually be recognized by cross-species hybridization. The cloning of a human gene usually leads directly to the cloning of a mouse homolog, and this can then be used for pharmacogenomic studies. Application of both in vitro and in silico approaches successfully resulted in the rapid identification of the basis for genetic variability in irinotecan, a chemotherapeutic agent. The drug’s metabolism in mice was found to mirror genetic variability in humans that contributes to interindividual differences in response to the medication (22).
III. Mouse Pharmacogenetic Approaches for Airway Diseases

Many mouse models are widely used in respiratory experiments. Inbred strains and genetic modified mice also can have important roles in pharmacogenetic and pharmacogenomic approaches for airway disease (Table 1).

Genetic mapping in inbred strains can identify loci that are associated with phenotype traits of interest. This strategy requires two strains of mice in which the phenotypic trait or traits of interest are significantly different, for example, at opposite ends of the distribution. Two studies have used this methodology to successfully localize the QTLs of bronchial hyperresponsiveness in mice (23,24). Susceptibility to acute lung injury by ozone has also been assessed, and the key loci identified (25).

Inbred mouse strains with different drug response characteristics could also be used to apply an inbred gene mapping approach to pharmacogenomics (21).

As inbred mice, genetically modified mice also are of great utility, including gene knockout, gene knockin, and transgenic mouse models. A knockout mouse is a genetically engineered mouse in which one or more genes have been made inoperative.
Knockout mice are important animal models for studying the role of genes that have unknown functions. The disadvantage of gene knockout is that it can cause potential lethal or developmental effects. For example, knockout of the \( GR \) gene was found to result in mouse death at the embryonic stage (26). To avoid this problem, a conditional knockout approach allows researchers to delete the gene of interest in a time- and space-dependent manner. Site-specific recombinase systems Cre-loxP and Flip-FRT are used to excise a critical part of the gene. Timing and tissue-dependence are achieved by the choice of promoter used to drive the Cre gene. The recombineering technique uses homologous recombination mediated by the lambda phage Red proteins to rapidly introduce loxP or FRT sites into the subcloned DNA to achieve conditional knockout (27).

An example of a drug target developed by knockout comes from the study of T-bet, a TH1-specific T-box transcription factor. T-bet transactivates the IFN-\( \gamma \) gene in TH1 cells and has the unique ability to redirect fully polarized TH2 cells into TH1 cells. T-bet knockout mice demonstrated a physiological and inflammatory phenotype in murine airways similar to that created by allergen exposure in sensitized mice, in the absence of an induced inflammatory response. This phenotype existed in naive mice spontaneously and was similar to that observed in mice following allergen sensitization and challenge. It suggested that T-bet might be an attractive target for the development of anti-asthmatic drugs (28).

A knockin mouse model is when the normal mouse gene is replaced by a mutant version of the mouse gene using homologous recombination allowing study of the variant gene. A transgenic overexpression model is when an allele of a human polymorphism is put into a mouse either with or without the mouse background. This technique enables the dissection of the pharmacogenetic effect of human polymorphisms. The transgenic overexpression model can also be made tissue-specific by the use of different promoters.

### Table 1

<table>
<thead>
<tr>
<th>Mouse model</th>
<th>Genotypes</th>
<th>Characteristics and usages</th>
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<tbody>
<tr>
<td>Knockout</td>
<td>( --/-- )</td>
<td>Loss of function, allowing dissection of unknown gene function</td>
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<tr>
<td>Conditional knockout</td>
<td>( --/-- )</td>
<td>Loss of function, allowing dissection of unknown gene function in time and space</td>
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<td>Transgenic:</td>
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<tr>
<td>Transgenic overexpression of human gene</td>
<td>( m/m, H/H )</td>
<td>Gain of function, allowing dissection of human gene function</td>
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<td>Transgenic overexpression of both human alleles</td>
<td>( m/m, H/h )</td>
<td>Gain of function, allowing dissection of function of human SNP allele</td>
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<tr>
<td>Knockout/knockin</td>
<td>( m^-/m^- )</td>
<td>Gain of function, allowing dissection of human gene function without the mouse homolog of the gene being present</td>
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<td>( H/H )</td>
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\( m/m \) and \( m^-/m^- \) represent mouse genotypes
\( H/H \) and \( H/h \) represent human genotypes

**Abbreviation:** SNP, single nucleotide polymorphism.
The overexpression of human allelic variants in separate lines of mice is a very useful tool to study human pharmacogenomic variants. An excellent example of this are the transgenic FVB/N mice containing human ADRB2. A threonine to isoleucine polymorphism at amino acid 164 in the fourth transmembrane spanning domain of the β2-adrenergic receptor (β2AR) is known to occur in the human population (29). To explore potential differences between the two receptors, transgenic mice were created that either overexpressed wild-type β2AR or the mutant Ile-164 receptor in a targeted manner in the heart using a murine α-myosin heavy chain promoter. The functional properties of the two receptors were then assessed at the level of in vitro cardiac myocyte signaling and in vivo cardiac responses in intact animals. Comparison of the expression levels of these receptors in the two mouse lines revealed an approximately 45-fold increase in β2AR expression in the mutant Ile-164 receptor transgenic mice in comparison to the wild-type overexpression mice. Myocyte membrane adenylyl cyclase activity in the basal state was significantly lower in the Ile-164 mice compared to wild-type β2AR mice. The Ile-164 polymorphism was substantially dysfunctional therefore in the relevant target tissue (30).

The opportunities with mouse models are expanding as new and relevant techniques are being developed such as vectors that allow knockin genes to be exchanged more readily—so-called gene-swapping. A knockout/knockin approach may be used to create mouse lines that express human allelic variants that can then be evaluated for their pharmacologic relevance. In these experiments, the mouse gene is removed and replaced with human major and minor allelic variants. This technique of “zygotic injection” means that mouse lines carrying numerous human alleles could be a reality in the very near future (31).

IV. Pharmacogenetics and Pharmacogenomics Research into Airway Diseases

To date, progress in pharmacogenetics and pharmacogenomics of airway diseases has predominantly focused on the targets of commonly used drugs such as β2-adrenergic agonists, corticosteroids, and leukotriene (LT) modifiers. However, only a limited number of few polymorphisms with pharmacogenetic roles have been identified and prospective clinical trials are on the way to validate the effects of these genetic variations in clinical practice.

A. β2-Adrenergic Agonists

β2-Adrenoceptor agonists (β-agonists) have been used for at least 5000 years to relieve bronchoconstriction (32). There are two general classes of β-adrenergic agonists. Short-acting β-agonists are used in the management of mild asthma to relax airway smooth muscle. Long-acting β-agonists are usually prescribed for moderate to severe persistent asthma or patients with COPD.

The β2AR is the target molecule of β2-agonists. The gene for the receptor, ADRB2, is located on chromosome 5q31. It is a small, intronless gene with 413 amino acid residues (33). The pharmacogenetic screen of ADRB2 began with the work of Liggett and his colleagues (34). It is a highly polymorphic gene with more than 50 SNPs within it and in close proximity to it (35). Three coding (nonsynonymous) polymorphisms have been identified in ADRB2: Arg16Gly, Gln27Glu, and Thr164Ile. The
two SNPs at amino acid positions 16 and 27 have been well characterized. Thr164Ile is a rare polymorphism with a minor allele frequency of 3% in the Caucasian population (36). Most clinical studies have therefore focused on the Arg16Gly SNP. Arg16 homozygotes had been found to have greater initial bronchodilator responses to \( \beta_2 \)-agonists than Gly16 homozygotes in response to short-acting \( \beta \)-agonists (37,38). However, following prolonged exposure to the short-acting \( \beta \)-agonists, greater downregulation was observed (39). There have been many other small retrospective reports of this particular polymorphism that have either confirmed the original findings or contradicted the original results with either opposite observations or no associations seen at all (40). In two randomized studies to test the Gly16Arg polymorphism and its effect on the response to long-acting \( \beta_2 \)-agonist therapy, no pharmacogenetic effect of \( ADRB2 \) variation on therapeutic response in asthma patients, irrespective of their Arg16Gly genotype, was observed (41). The Gln27Glu polymorphism has been found to be associated with the response to \( \beta_2 \)-agonist treatment in children during an asthma exacerbation. Homozygous Gln27 patients responded less effectively to treatment with inhaled \( \beta_2 \)-agonists during an acute asthma exacerbation (42).

The fundamental question of the pharmacogenetic role of the \( ADRB2 \) locus, in particular the nonsynonymous amino acid changes, remains somewhat uncertain. LD is strong across the region, indicating that other variants nearby may be involved. The next pharmacogenetic approach for the \( ADRB2 \) locus would be the implementation of carefully designed prospective studies of asthmatic patients with short-acting or long-acting \( \beta_2 \)-agonist treatments.

\( Arginase \) I (\( ARG1 \)) is another gene that has recently been identified as a potential novel bronchodilator response gene (43). The gene is located on chromosome 6q23 and has 8 exons that through alternative exon splicing result in 10 splice variant transcripts. Three of these splice variants translate into proteins. The transcript length of the gene is 1532 bps and it encodes 322 amino acids. The \( ARG1 \) protein targets L-arginine. L-arginine is involved in nitric oxide synthase to form an endogenous dilator nitro oxide (NO) (44). Arginase (\( ARG1 \)) is postulated to be involved in asthma by deleting stores of L-arginine, which leads to decreased production of NO. This process results in inhibition of smooth muscle relaxation (45).

In a study of 844 SNPs from 111 candidate genes selected from \( \beta \)-agonist and corticosteroids pathways, three \( ARG1 \) SNPs were associated with inhaled bronchodilator after long-acting \( \beta_2 \)-agonist treatment. One SNP rs2781659 was significantly associated with bronchodilator in the four population association study. Homozygote A/A individuals of rs2781659 were found to have a greater response to \( \beta_2 \)-adrenergic agonists than homozygote G/G individuals (43). The functions of the variant remain unknown, but all three associated \( ARG1 \) SNPs were in the promoter region of the gene and were in tight LD with each other. Genotyping of all known SNPs in the gene or resequencing of the gene will need to be performed to determine if these three promoter SNPs are in LD with any of the four nonsynonymous SNPs previously identified in the gene. Studies of human asthma bronchoalveolar lavage cells have shown elevation of the \( ARG1 \) protein (45) while mouse studies have shown that \( ARG1 \) expression is increased in the murine asthma lung. Further studies in mice in which RNAi for arginase 1 in the lungs has been performed resulted in a loss of airway hyperresponsiveness to methacholine (46).
B. Corticosteroids

Corticosteroids are the most effective and commonly used drugs for the treatment of asthma and COPD. They bind to the intracellular GR after which the complex translocates to the nucleus where it is involved in the regulation of gene expression (47). The response to ICSs in patients with asthma or COPD is highly variable. Some subjects are deemed unresponsive since they fail to react to high doses of inhaled or even oral glucocorticoids (GCs) (48,49). GC-resistant patients have been shown to have abnormalities in the activity of proinflammatory transcription factors AP1 and NF-κB. These two factors are important for the induction of transcription of a number of chemoattractants, cytokines, cytokine receptors, and cell adhesion molecules (50).

To evaluate the gene expression profiling of GC-sensitive and GC-resistant asthmatic patients, Hakonarson et al. applied gene microarrays, allowing expression measurement of 11,812 genes, using as substrate blood mononuclear cells at baseline and poststimulation with IL-1β/TNF-α with or without dexamethasone. The expression profile of 923 genes was significantly reversed in GC responders in the presence of GC. Fifteen genes were found to predict GC response on extreme phenotypes (51). The results, although not definitive, provide an interesting resource for the future pharmacogenetic investigations.

A few candidate genes have been implicated in the response to corticosteroids. The GR gene (also known as NR3C1) belongs to a large family of nuclear receptors. GR gene is located on chromosome 5q31 and contains nine exons that encode for a 778 amino acid peptide of which there are five splice variants. There are two naturally occurring isoforms of the GR gene: GRα (functional) and GRβ (no hormone-binding ability). A total of 15 missense, 3 nonsense, 3 frameshift, 1 splice site, and 2 alternative spliced mutations have been reported in the GR gene as well as 16 SNPs in noncoding regions, and many have shown association with GC resistance (52). One polymorphism Asp363Ser, although rare, may nonetheless be of importance. In a study of 216 elderly patients, 13 heterozygotes for the Asp363Ser site were identified. Individuals carrying 363Ser polymorphism seemed healthy at clinical examination but had a higher sensitivity to exogenously administered GCs, with respect to both cortisol suppression and insulin response (53).

CRHR1 is a major regulator of GC synthesis. It plays a major role in the stress response through its regulation of endogenous GC and catecholamine production. In the absence of the CRHR1 ligand, it was found that endogenous GC and catecholamine production decreased, resulting in allergen-induced airway inflammation and lung mechanical dysfunction (54). The CRHR1 gene is located on human chromosome 17q21.3 and it has 14 exons that encode 447 amino acids. Two splicing variants of CRHR1 have been identified. Three haplotype-tagging SNPs (rs1876828, rs242939, rs242941) in CRHR1 were found to be associated with lung function improvement after eight weeks’ treatment with ICSs in patients with asthma. Individuals homozygous for the haplotype GAT/GAT had an enhanced response to glucocorticoids (55,56). However, association of the three CRHR1 SNPs variants with long-acting β2-agonists response was not observed in an asthma cohort study that spanned 22 years (57).

The genome structure of the chromosome 17q21.3 region is interesting since within it there is a 900-kb inversion contained within a large region of conserved LD and also containing the CRHR1 gene. It is therefore conceivable that the CRHR1 response to
ICSs may be as a result of natural selection resulting from inversion status or by long-range LD with another gene (15) and is certainly worthy of further investigation.

T-box 21 (TBX21) encodes T-bet, a transcription factor crucial for naïve T-lymphocyte production that influences naïve T-lymphocyte development and has been implicated in asthma pathogenesis. The T-bet knockout mouse spontaneously develops airways inflammation and hyperresponsiveness suggestive of asthma (28). TBX21 is located on chromosome 17q21.32 and it has six exons encoding 535 amino acids. In a large clinical trial spanning over four years, a nonsynonymous variation in TBX21, coding for replacement of histidine with glutamine at aa position 33, has shown association with significant improvement in the PC(20) of asthmatic children. This increase enhanced the overall improvement in PC(20) that was associated with ICS usage (58). TBX21 may therefore be an important pharmacogenetic determinant of response to the therapy of asthma with ICSs.

A novel variant in FCER2, which encodes for the low-affinity immunoglobulin E (IgE) receptor, has recently been associated with asthma exacerbation and ICSs. The gene is located on chromosome 19p13 having 11 exons that encode 321 amino acids. In a study of the relationship between polymorphisms of FCER2 and severe exacerbations in children with asthma, three FCER2 SNPs were significantly associated with elevated IgE levels at age 4. Each SNP was also found to be associated with increased severe exacerbations. Using multivariable models, associations between one novel mutation T2206C and severe exacerbations in both white and African-American children were seen. The T2206C SNP was associated with increased risk of exacerbations in asthmatic children taking ICSs despite the protective nature of this medication with regard to exacerbations (59). The novel variant was also associated with higher IgE levels, suggesting that differential expression in FCER2 can adversely affect normal negative feedback in the control of IgE synthesis and action (39).

C. LT Modifiers

LT modifiers inhibit the action of LTs, which are a family of lipoxygenated eicosatetraenoic acids derived from the metabolism of arachidonic acid. They have been shown to be produced in the airways where they act as potent bronchoconstrictors. Many enzymes are involved in the processing of the LTs. Two classes of LT modifier are available for use in the management of asthma: 5 lipoxygenases (5-LO) inhibitors and LT receptor antagonists, for example, montelukast and zafirlukast.

The polymorphisms of several genes have been associated with altered responses to LT modifiers and inhibitors. The ALOX5 gene is located on chromosome 10q11. It contains 14 exons and encodes 674 amino acids. To date, 14 SNPs have been identified in the transcript. In a study of the 5-lipoxygenases inhibitor ABT-761, response to treatment was shown to be related to genotypes of a VNTR in the promoter region of the ALOX5 gene (14). Further studies however did not confirm these initial findings.

Another 5-lipoxygenase inhibitor called montelukast was associated with a 73% reduced risk of an asthma exacerbation in carriers of mutant allele as compared with homozygous wild type (5/5 repeats) (60), which was not consistent with the original report (14). In another somewhat smaller study, montelukast treatment decreased the number of asthma exacerbations in patients with 5/5 or 4/5 repeats but not in participants who were 4/4 homozygotes for the VNTR (61). In a study of the VNTR polymorphism and asthma severity, asthmatics with non5/non5 genotype expressed less ALOX5.
mRNA and produced less LTC4 into culture supernatants than 5/5 individuals while asthmatic children bearing non5/non5 genotype had more moderate-severe asthma than children with the 5/5 genotype. Multivariate logistic regression identified \( ALOX5 \) promoter genotype as a significant predictor of disease severity. Children bearing the non5/non5 genotype had a greater response to exercise as measured by the maximum fall after exercise and the area under the exercise curve (62). These confusing results indicate that the INDEL variant in the promoter of \( ALOX5 \) needs to be studied in larger and more diverse populations with asthma to validate the function role in LT modifiers treatment.

LT C4 synthase (LTC4S) and LT A4 hydrolase (LTA4H) are enzymes of the LT pathway. The gene \( LTC4S \) is located on human chromosome 5q35.3 and has five exons that encode 150 amino acids. Five splice variants of the gene exist, and to date three SNPs have been identified in the gene. A promoter SNP A-444C has been reported to be associated with severe asthma (63). Three independent reports suggested an association between the response to long-acting \( \beta \)-agonists and the C allele of the same promoter polymorphism (64–66). The gene \( LTA4H \) is located on 12q23 and has 19 exons and encodes 611 amino acids. Individuals homozygote for the G allele of the SNP rs2660845 in \( LTA4H \) exhibit a four- to fivefold increased probability of having an asthma exacerbation while receiving montelukast treatment comparing with individuals homozygous for the A allele (60).

\( SLCO2B1 \) (solute carrier organic anion transporter family, member 2B1) codes OATP2B1, a mediated transport of montelukast. This gene is located on chromosome 11q13. It has four exons that encode 709 amino acids. A nonsynonymous SNP (rs12422149, Arg312Gly) within the gene has been shown to be associated with symptom improvement scores after montelukast treatment. Patients with the Arg/Arg genotype significantly exhibited a reduced plasma concentration on the morning after an evening dose of montelukast as well as the observation of a significant improvement in symptoms (67). The relatively small sample size is however a limitation of this study. Consequently, more studies involving larger sample sizes will be needed to accurately access the associations between the Arg312Gly polymorphism and responsiveness to montelukast treatment.

\( CYSLTR2 \) encodes the CysLT2 receptor, which is involved in the LT pathway, has a greater response to LT modifiers, and plays an important role in smooth muscle cell proliferation. The gene is located on chromosome 13q14 and has a single exon that encodes a protein 346 amino acids in length. Two SNPs (rs912278, rs912277) within the gene have been reported to be associated with the montelukast. The common \( CYSLTR2 \) TT and TC haplotypes of SNPs rs912278 and rs912277 have both been shown to have a significantly lower mean change in morning PEF observations that are consistent with the single-marker results (68).

D. Theophylline

TP and other methylxanthine derivatives have weak bronchodilator and anti-inflammatory effects that have been used in both COPD and asthma management. TP has a narrow therapeutic range because of its dose-related toxicities. The plasma concentrations of some patients have been shown to have the potential to reach the toxic range (over 20 \( \mu g/mL \)) despite the fact that patients were only prescribed 400 mg/day of a slow-release TP and were administered no other medications and had no disease
complications. Cytochrome P450 (CYP) 1A2 is the major metabolic enzyme of TP. The gene (CYP1A2) is located on chromosome 15q24.1 and has 7 exons encoding 516 amino acids. A common promoter polymorphism –2964G/A of the gene is associated with altered clearance of TP. The clearance of TP in patients with the A/A genotype was markedly reduced when compared with patients possessing the G/G genotype. This genetic variation could be helpful in predetermining individuals (A/A homozygotes for –2964G/A) who may be sensitive to TP toxicity despite being described standard routine doses of slow-release TP (69).

Most of the pharmacogenetic loci for airway diseases detailed above (Table 2) were identified in studies involving relatively small sample sizes. A consistent theme from all of these studies is that next key pharmacogenetic approach would be to verify these potentially functional polymorphisms in larger population sets.

E. Other Treatments

Antihistamine, anticholinergic, and anticytokine treatments are all further types of management implemented in the treatment of airway diseases.

Histamine is a bronchoconstrictor that is involved in the pathogenesis of airway disease. In the bronchial epithelium, histamine N-methyltransferases (HNMTs) play an important role in histamine biotransformation by catalyzing the methylation of histamine. The levels of HNMT activity in human tissues are controlled, in part, by genetic variation. A common C314T polymorphism within the \textit{HNMT} gene results in a Thr105Ile change in the encoded amino acid at position 105 (nucleotide position 314), and the T314 allele is associated with decreased levels of both HNMT enzymatic activity and immunoreactive protein. Presence of the T314 allele would therefore be anticipated to result in reduced histamine metabolism and increased bronchoconstriction. In an association study of this polymorphism with asthma patients, T-allele frequency was found to be significantly higher in asthma patients. This suggests that individual variation in histamine metabolism might contribute to the pathophysiology and/or response to therapy of this disease (70).

Anticholinergic agents are substances that block the neurotransmitter acetylcholine in the central and the peripheral nervous system. The majority of anticholinergic drugs are antimuscarinics. Antimuscarinic agents are effective as bronchodilators in treatment of both COPD and asthma. Ipratropium bromide is a nonselective antimuscarinic agent that acts as an antagonist at M1, M2, and M3 receptors. The genes encoding the receptors are therefore potential targets for a pharmacogenetic study. The coding regions for the M2 and M3 receptor genes (\textit{CHRM2} and \textit{CHRM3}) have already been screened for polymorphic variants, and while both aa coding regions of the two genes contain SNPs, the level of expression of the M2 and M3 receptors in the airways is partly driven by a transcriptional controlling mechanism. Both \textit{CHRM2} and \textit{CHRM3} promoters have been defined and are known to contain a range of polymorphic variants, but until now no evidence has been found for any association between the promoter polymorphisms and drug response (34).

Anticytokines are quite new and novel therapies for asthma and COPD. T\textsubscript{h}2 cytokines are recognized to play an important role in airway inflammation. IL-4 and IL-13 are critical cytokines in the expression of atopy and allergic disease. IL-4 triggers B lymphocytes to produce IgE, induces the expression of adhesion molecules on endothelium that specifically attract eosinophils, and triggers T cells to become T\textsubscript{h}2 cells,
### Table 2  Potential Pharmacogenetic Loci for Airway Diseases

<table>
<thead>
<tr>
<th>Gene</th>
<th>Name</th>
<th>Chromosome location</th>
<th>Pharmacogenetic polymorphism allele</th>
<th>Drug target</th>
<th>Associated phenotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADRB2</td>
<td>β2-Adrenergic receptor</td>
<td>5q31.32</td>
<td>Arg16Gly</td>
<td>β2-Adrenergic agonists</td>
<td>Arg/Arg homozygotes have greater response to β2-agonists</td>
</tr>
<tr>
<td>ARG1</td>
<td>Arginase I</td>
<td>6q23</td>
<td>rs22781659</td>
<td>β2-Adrenergic agonists</td>
<td>A/A homozygotes have greater response to β2-agonants</td>
</tr>
<tr>
<td>GR</td>
<td>Glucocorticoid receptor</td>
<td>5q31</td>
<td>Asp363 Ser</td>
<td>Corticosteroid</td>
<td>363Ser have an enhanced response to glucocorticoids</td>
</tr>
<tr>
<td>CRHR1</td>
<td>Corticotropin-releasing hormone receptor 1</td>
<td>7q21.3</td>
<td>Haplotype</td>
<td>Corticosteroid</td>
<td>GAT/GAT homozygotes have an enhanced response to glucocorticoids</td>
</tr>
<tr>
<td>TBX21</td>
<td>T-box 21</td>
<td>17q21.3</td>
<td>His33Gln</td>
<td>Corticosteroid</td>
<td>33Gln have an enhanced response to glucocorticoids</td>
</tr>
<tr>
<td>FCER2</td>
<td>Fc fragment of IgE, low affinity II, receptor</td>
<td>19p13.2</td>
<td>T2206C</td>
<td>Corticosteroid</td>
<td>T/T homozygotes have an enhanced response to glucocorticoids</td>
</tr>
<tr>
<td>ALOX5</td>
<td>Arachidonate 5-lipoxygenase</td>
<td>10q11.2</td>
<td>Promoter VNTR</td>
<td>Leukotriene modifiers</td>
<td>Mutant homozygotes have decreased 5-lipoxygenase inhibitor</td>
</tr>
<tr>
<td>LTC4S</td>
<td>Leukotriene C4 synthase</td>
<td>q35.3</td>
<td>Promoter A-444C</td>
<td>Leukotriene modifiers</td>
<td>-444C has greater response to leukotriene modifiers</td>
</tr>
<tr>
<td>LTA4H</td>
<td>Leukotriene A4 hydrolase</td>
<td>12q23</td>
<td>rs2660845</td>
<td>Leukotriene modifiers</td>
<td>A/A homozygotes have greater response to leukotriene modifiers</td>
</tr>
<tr>
<td>CYSLTR2</td>
<td>Cysteinyl leukotriene receptor 2</td>
<td>13q14</td>
<td>rs91227/rs912278</td>
<td>Leukotriene modifiers</td>
<td>Haplotype CC has greater response to leukotriene modifiers</td>
</tr>
<tr>
<td>SLC02B1</td>
<td>Solute carrier organic anion transporter family, member 2B1</td>
<td>11q13</td>
<td>Arg312Gly</td>
<td>Leukotriene modifiers</td>
<td>Arg312 homozygotes have greater response to leukotriene modifiers</td>
</tr>
<tr>
<td>CYP1A2</td>
<td>Cytochrome P450, family 1, subfamily A, polypeptide 2</td>
<td>15q24.1</td>
<td>−2964G/A</td>
<td>Theophylline</td>
<td>Heterozygotes G/A reduce clearance of theophylline</td>
</tr>
</tbody>
</table>
which in turn further release IL-4 and mediate allergic diseases. IL-13 is a cytokine closely related to IL-4 that binds to IL-4R and is also expressed by Th2 cells from asthma patients (71). Genetic regulation of IL-4 and IL-13 is an important pivot in the genetic predisposition to allergy. Administration of either IL-13 or IL-4 confers an asthma-like phenotype, by an IL-4 receptor chain-dependent pathway, in nonimmunized T cell-deficient mice (72).

For IL-4 blockade, two vaccines have been tried in mice. One is limpet hemocyanin (73), an IL-4 derivative immunogen. The other is a 14–amino acid peptide from IL-4 inserted into the variant hepatitis B core antigen (74). Both vaccines induced high antibody titers in mice and inhibited antigen-induced lung inflammation. IL-13 not only binds to the low-affinity IL-13α1 subunit but also binds to the high-affinity complex formed by the IL-13 α1 and IL-4 α1 subunits. Binding of the latter leads to the activation of Janus kinase1 (JAK1) and JAK2, and the signal transducer and activator of transcription STAT6 (75). A nonsignaling high affinity IL-13Rα2 was shown to strongly inhibit the activity of IL-13 in mice and in humans (76). In humans, an IL-13-specific mAb CAT-354 in mild asthma has been used in phase 2 studies (75). STAT6 is the transcription factor for both IL-4 and IL-13. In human, the polymorphisms of the gene are associated with asthma (77,78). A study involving local application of cell-penetrating peptide inhibitors (a dormant-negative novel chimeric peptide) of STAT-6 showed significant promise with regard to the treatment of allergic rhinitis and asthma (79).

In addition to IL-4 and IL-13, other cytokines play crucial roles in airway diseases. IL-5 and IL-9 are the major cytokines that drive eosinophil differentiation while IL-10 and IFN-γ inhibit multiple inflammatory cytokines. IL-12 induces naïve T cells into the Th1 pathway. TNF-α is another Th1 pathway simulator. There is strong evidence that polymorphisms of many of the genes encoding these cytokines as well as those encoding IL-15 and IL-17F are associated with human airway diseases traits. Inhibition of single cytokines may not be sufficient in the treatment of airway disease, and there needs to be elucidation of the function of the variants underlying the associations that have been seen has. Nonetheless, these cytokines are potential therapeutic targets in airway disease and some clinical trials are already in progress (Table 3).

V. The Future of Pharmacogenetics and Pharmacogenomics of Airway Diseases

Although there are already a number of genetic studies that have identified functional polymorphisms of relevance to drug targets and drug responses, prospective clinical studies of these polymorphisms and the genes they are in have been relatively small. Most studies have been retrospective and have involved small numbers of patients, and have been focused on specific polymorphisms without systematic representation of the variation in the genes that are under study (4).

The field of genetics has the opportunity of identifying new associations in novel genes for airway diseases. To date there have been more than 500 papers published of disease-gene studies (predominantly candidate gene studies) for asthma, with over 100 genes reportedly being associated with asthma phenotypes (113). Whole genome linkage screens have identified new loci for asthma (114) and COPD (115) that following fine mapping and positional cloning has ultimately resulted in disease gene identification.
<table>
<thead>
<tr>
<th>Gene</th>
<th>Chromosome location</th>
<th>Traits gene polymorphisms associated with humans</th>
<th>Animal models established for the purpose of drug development</th>
<th>Clinical trial approaches</th>
<th>Predicted effects of novel therapeutic targets</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-4</td>
<td>5q31</td>
<td>Asthma (80); IgE (81)</td>
<td>Vaccines in mice (73, 82)</td>
<td>Inhaled IL-4 receptor (84)</td>
<td>Blocking IL-4 Th2 phenotype</td>
</tr>
<tr>
<td>IL-13</td>
<td>5q31</td>
<td>Asthma (85); IgE (86)</td>
<td>mAb in mice (87)</td>
<td>mAb in monkey (72)</td>
<td>Blocking IL-13 Th2 phenotype</td>
</tr>
<tr>
<td>IL-5</td>
<td>5q31</td>
<td>Eosinophil counts (89)</td>
<td>Administration of IL-12 in mice (94)</td>
<td>Administration of rhIL-12</td>
<td>Blocking eosinophil differentiation</td>
</tr>
<tr>
<td>IL-10</td>
<td>1q32</td>
<td>Asthma (92); COPD (93)</td>
<td>Administration of IL-10 in mice (95)</td>
<td>Administration of rhIL-10</td>
<td>Inhibiting inflammatory cytokines</td>
</tr>
<tr>
<td>IL-12/A/B</td>
<td>3q25/5q33</td>
<td>Asthma (102), IgE (103); COPD (104)</td>
<td>Overexpressing IL-12 in mouse dendritic cell (97)</td>
<td>Administration of rhIL-12</td>
<td>Boosting Th1 pathway</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>12q14</td>
<td>Asthma (102)</td>
<td>Administration of IFN-γ in mice (100)</td>
<td>Administration of rhIFN-γ</td>
<td>Suppressing Th2 inflammation</td>
</tr>
<tr>
<td>TNF-α</td>
<td>6p12.2</td>
<td>Asthma (102, 103); COPD (104)</td>
<td>Administration of anti-TNF in mice (105)</td>
<td>Administration of infliximab in mice (106)</td>
<td>Anti-inflammatory</td>
</tr>
<tr>
<td>IL-15</td>
<td>4q31.21</td>
<td>Asthma (102), IgE (103)</td>
<td>Soluble IL-15 in mice (109)</td>
<td>Administration of anti-IL-15</td>
<td>Suppression of Th2 inflammation</td>
</tr>
<tr>
<td>IL-17F</td>
<td>6p12.2</td>
<td>Asthma (111)</td>
<td>Pravastatin in mice (112)</td>
<td>Administration of anti-IL-17</td>
<td>Anti-IL-17 mediating inflammation</td>
</tr>
</tbody>
</table>

Pitrakinra, an IL-4 mutant protein; mepolizumab, humanized blocking IgG mAb; infliximab, anti-tumour necrosis factor (TNF)-α mAb; etanercept, TNF receptor fusion protein; Mibefrudil, monoclonal antibody identifies CD122, the β-subunit shared by the IL-2 and IL-15 receptor.

Abbreviation: mAb, monoclonal antibody.
These genes include ADAM33 (a disintegrin and metalloproteinase-33) on chromosome 20p13 (49), DPP10 (dipeptidyl peptidase-10) on chromosome 2q14 (116), PHF11 (plant homeodomain finger protein 11) on chromosome 13q14 (117), GPR A (G protein-coupled receptor for asthma) on chromosome 7p14 (118), HLA-G on chromosome 6p21, and CYFLP on chromosome 5q33 (119). Both candidate and positional cloning approaches have in some instances identified genes that could be useful targets for pharmaceutical intervention.

To date there has only been one GWA study for asthma, which has resulted in the gene ORM DL3 (and potentially the neighboring GSDMLI gene) being identified as an important risk factor for the development of childhood asthma (120). Disease gene identification was aided by analyses of ORM DL3 transcript levels through genome-wide gene expression data from Epstein-Barr virus-transformed lymphoblastoid cells from children in the genotyped family samples (121). Little is known about the function of ORM DL3, which is a member of a conserved family of endoplasmic reticulum membrane proteins. It remains to be established whether ORM DL3 is a suitable target for drug development, although the protein contains four transmembrane regions and has the potential to act as a druggable transporter (122).

α1-Antitrypsin deficiency remains a paradigm for COPD and is still the most important known genetic risk factor for COPD (123). Mutations in the serpin peptidase inhibitor, clade A (α-1 antiproteinase, antitrypsin), member 1 (SERPINA1) gene cause α-1 antitrypsin deficiency. Numerous of other candidate genes could also be linked to disease pathogenesis. However, the candidate gene approach is often limited by inconsistent results in other study populations. The association of following genes with COPD were confirmed by different studies: transforming growth factor-β1 (TGFB1), surfactant protein B (SFTPB), serpin peptidase inhibitor clade E member 2 (SERPINE2), and microsomal epoxide hydrolase (EPHX1) (124). Interestingly, many of the genes identified by genome-wide linkage and/or association studies for asthma and COPD are expressed in the mucosa and respiratory epithelium, for example, DPP10 and GPR A, indicating that events at epithelial-cell surfaces might be driving disease processes. Understanding events at the epithelial-cell surface might provide new insights for the development of new treatments for inflammatory epithelial disease (125).

VI. Conclusions

In recent years, considerable progress has been made in the identification of pharmacogenetic and pharmacogenomic loci for airway diseases, but nonetheless we are still in the early stages of our understanding of the genetic effects on drug targets, metabolism, and disposition. With the available sequence information, including the intragenic noncoding regions of the human genome, the increasingly recognized regulatory microRNA profiles, the completion of sequences of human, mouse, and other species genomes, the establishment of different animal models and with advances in bioinformatics and methods to allow rapid genome-wide sequencing, genotyping, and expression studies, it is possible that more pharmacogenetic and pharmacogenomic loci for asthma and COPD genes will emerge soon. Translation of these findings into therapeutics for airway diseases will take time, but it is conceivable that improved patient care will be possible in the not too distant future (126).
References


BOOK: Chung_H7000  
CHAPTER 4  

TO: CORRESPONDING AUTHOR

AUTHOR QUERIES - TO BE ANSWERED BY THE AUTHOR

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| Q2 | Page: 126 AU: Please suggest if the bold face letters in Table 1 can be changed to normal face. |
| Q3 | Page: 128 AU: Please check the changes made in the sentence “l-arginine is involved in nitric oxide…” for clarity. |
| Q4 | Page: 130 AU: “zafirlister” changed to “zafirlukast” in the sentence “Two classes of LT modifier…” OK? |
| Q5 | Page: 134 AU: Please check the sentence “Inhibition of single cytokines may…” for clarity. |
Chapter: 4

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