Published in final edited form as:

Pediatr Emerg Care. 2006 January ; 22(1): 71–78.

Understanding Genomics: *Implications for the Emergency Medicine Physician and the Treatment of Asthma*

Robert J. Freishtat, MDMPH^{*} and Stephen J. Teach, MDMPH[†]

^{*} Assistant Professor of Pediatrics and Emergency Medicine, Division of Emergency Medicine, Children's National Medical Center, Washington, DC

[†] Associate Chief of Emergency Medicine, Children's National Medical Center, Washington, DC

Abstract

TARGET AUDIENCE—Physicians, nurse practitioners, and physician assistants who evaluate and care for children with minor illnesses.

Specialists including pediatricians, emergency physicians, pediatric emergency physicians, family practitioners, and pediatric nurse practitioners will find this information particularly useful.

LEARNING OBJECTIVES—After completion of this article, the reader will be able to:

1. Explain the role of single-nucleotide polymorphisms and haplotypes in the development of asthma and asthma exacerbations.
2. Describe what is currently known about how environmental influences interact with genotype to produce an asthmatic phenotype.
3. Describe the 3 possible effects of a single-nucleotide polymorphism or haplotype on pharmacology in the context of asthma.

Keywords

gene; asthma; linkage; microarray

In 2015, a 7-year-old African-American boy presents in your emergency department with a 2-day history of cough and wheeze. The symptoms began in the setting of a mild upper respiratory tract infection and were worsened by exercise. He is known to have chronic asthma as do his father and younger sister. His respiratory rate is 32 breaths per minute, and he is wheezing bilaterally and diffusely with moderate retractions. How will your evaluation and management begin? If recent trends in other fields are predictive, you will begin with a review of your patient's genetic profile.

Although that profile may guide a portion of or your entire approach, some facts about asthma will remain the same. It will still be a multifactorial chronic disorder,^{1–3} highly prevalent among children and adolescents,^{4,5} with a strong environmental component^{1,2,4,6–8} and genetic predilection.^{9–11} In addition, the pathophysiology will likely not have changed. As Sir William Osler wrote more than 100 years ago in *The Principles and Practice of Medicine*, “Asthma is a term which has been applied to various conditions associated with

Address correspondence and reprint requests to Robert J. Freishtat, MD, MPH, Research Center for Genetic Medicine, Children's National Medical Center, 111 Michigan Avenue, NW, Washington, DC 20010. E-mail: rfreishtat@cnmcresearch.org.. Dr. Freishtat has disclosed that he has no significant relationship with or financial interests in any commercial companies that pertain to this educational activity.

Dr. Teach has disclosed that he is/was a member of the speakers bureau of Astra Zeneca.

Wolters Kluwer Health has identified and resolved all faculty conflicts of interests regarding this educational activity.

dyspnea. Of the numerous theories, the following are most important: (1) that it is due to spasm of bronchial muscles, (2) that the attack is due to swelling of the bronchial mucous membrane, (3) and that, in many cases, it is a special form of inflammation of the smaller bronchioles."¹²

During the past century, our understanding of the pathophysiology of asthma has advanced slowly, without significant change in Sir Osler's basic paradigm. Now, however, with a completed "map" of the human genome in hand, new developments in the field of asthma pathobiology are arriving more quickly and often involve the identification of specific "asthma genes." Some of these asthma genes hold promise as potential predictors of the clinical variability of disease and even response to therapy. The advanced technologies used to make these discoveries will continue to slowly find their way into clinical practice.

PRIMER ON THE NEW GENOMICS

DNA as the Precursor of Phenotype

Genomic DNA, the genotype, is the chemical code for the assembly of protein via a RNA intermediate. DNA is a long series of triplets of various combinations of 4 nucleotides (adenine, thymine, cytosine, and guanine). Each triplet, a codon, is transcribed into RNA and then translated into 1 of 22 amino acids that are ultimately assembled to form proteins.

DNA is the basis for the hereditary passage of traits from parent to child, but misperceptions persist that a given genotype directly leads to a given phenotype. In reality, there are 3 components to phenotypic development: genotype, environmental influences, and the interaction between the two. This is the basis for the multifactorial etiology for asthma and other similarly complex diseases, which greatly complicates their study.

Mutations Versus Single-Nucleotide Polymorphisms

Phenotypic differences between individuals stemming solely from genotypic changes are attributed to spontaneous alterations or deletions in one or more base pairs of DNA. When one of these changes can be found in only 1% or less of the population and when it is *deterministic* (ie, necessarily results in phenotypic modification), it can be classified as a mutation. As causative of phenotypic change, a mutation must occur in coding regions (exons) of DNA. Because of the close relationship of the mutation with the disease, family pedigrees and chromosomal analysis allow for fairly routine identification of the causative genetic error.

Far more frequently, alterations in a single nucleotide occur in more than 1% of the population and cannot be identified as singly causative of any illness or trait. Unlike mutations, these changes can be found throughout the human genome, both in coding (exons) and noncoding (introns) regions of DNA. These are called single-nucleotide polymorphisms (SNPs), and because of their distribution throughout the genome, they are *probabilistic* (ie, do not always lead to phenotypic variation). For example, the nucleotide change may occur in a noncoding region of DNA or may not ultimately alter the translated amino acid because multiple codons can denote a single amino acid (Fig. 1). However, many of more than 3 million recorded SNPs do have known or suspected functionality in various diseases, including several for asthma.¹³

Overall, SNPs are relatively frequent, occurring once every 300 base pairs.¹³ To date, there are estimated to be approximately 11 million SNPs in the approximately 3 billion base pairs of the human genome, making them the most common form of human genetic variation.¹⁴ Their frequency, however, is variable according to race and ethnicity.¹⁵ Evidence suggests that approximately half of SNPs are silent, meaning that there is ultimately no change in the amino acid coded for by the SNP-containing codon.¹⁶ In addition, most SNPs in humans are unequally distributed through the genome. This is the notion of linkage disequilibrium. This concept derives from the tendency for closely approximated SNPs in one region of a gene to

remain clustered together over many generations because their close proximity decreases their chances of being separated when genes recombine during fertilization. Each variant of these clusters (eg, AATGGTGT vs ACTGGTAT) is called a haplotype and represents one possible combination of SNPs in a region of a gene. Thus, although there are 3 million identified SNPs, because of the frequency of linkage disequilibrium, there are only 300,000 identified haplotypes.¹³

Genomewide Linkage Analysis and Association Studies

The standard theory underlying the asthmatic phenotype is that many genes, both beneficial and detrimental, interact with each other and the environment, ultimately leading to one of several asthmatic phenotypes. To better understand the contribution of specific genes toward the development and response to treatment of asthma, researchers can undertake linkage analysis studies, which are essentially pedigree studies looking at affected and unaffected family members. The affected family members' genotypes are examined for markers that track through their pedigree but do not track through that of the unaffected members. Log-of-the-odds scores determine which markers (ie, SNPs and/or haplotypes) are most likely to cluster among affected members, and in turn, those markers may be located proximally to genes that are important in asthma pathogenesis.

After the identification of potentially biologically significant candidate gene segments, an association study (essentially a case-control investigation) can be used to determine the specific shared DNA regions among affected individuals. In this type of study, matched asthmatic and healthy controls are compared for prevalence of specific SNPs and haplotypes. A more prevalent SNP or haplotype in the asthmatics suggests a higher risk of asthma among patients with that genotype.

Using these techniques, there are now several successful genomewide linkage studies for asthma that have identified a number of chromosomal regions as containing possible candidate genes for roles in asthma pathogenesis, the so-called "asthma genes."^{7,17,18} These include chromosome 5q, chromosome 11q (β chain of Fc ϵ RI), chromosome 12q (interferon γ , STAT-6), and chromosome 16p (interleukin 4R).¹⁹

Incorporation of Environmental Factors

Although these genomic advances have identified potential "asthma genes," environmental influences will continue to interact with these genotypes to determine the pathogenesis of asthma. Because patients and their environmental exposures vary widely, these interactions have been difficult to study. However, a recent publication identified 3 regions of DNA in a genomewide linkage analysis study that may contain candidate genes for the development of asthma in patients with environmental tobacco smoke exposure: regions of chromosome 1p, chromosome 5q, and chromosome 9q.⁶ In addition, other genes with potential interaction with tobacco smoking and risk for asthma exacerbations have been identified in candidate gene linkage approaches:²⁰ interleukin 10,²¹ interleukin 1 β ,²² and matrix metalloproteinases 1 and 12.²³

RNA as the Mediator of Phenotype: Microarray Technology

Although DNA holds the information for cellular construction and processes, it is RNA that mediates the transition between DNA and proteins, where phenotype is expressed. As such, the study of RNA expression using nucleic acid sequence microarrays made them the first genomewide high-throughput genomic technologies to undergo widespread use by scientists in many areas of biology.

The basis for microarray technology is that messenger RNA (mRNA) from study tissue is reverse transcribed into complementary DNA and then transcribed back into fluorescently labeled complementary RNA before being hybridized (ie, annealed by a chemical- and heat-induced reaction) to millions of pieces of RNA (oligonucleotide probe sets) which have been bound onto a glass slide in an array pattern. The degree of hybridization is measured by a signal detection apparatus and computer software allowing for the calculation of the original relative concentration of mRNA in the study tissue (Fig. 2). These genome-anchored arrays are available for many species, including human, mouse, and rat, enabling their use in animal models of asthma.

The expense of preparation and analysis of microarrays has prompted a cooperative approach to data resulting from these studies. Most journal-published microarray data are Web-accessible, and much of it searchable and/or analyzable on the Web by researchers without access to specialized equipment in their laboratories. This has been the case since the Minimal Information About a Microarray Experiment guidelines (<http://www.mged.org>) were presented in 2001.²⁴ An example of this is the Public Expression Profiling Resource portal (<http://pepr.cnmcresearch.org/home.do>) of the Research Center for Genetic Medicine at Children's National Medical Center in Washington, DC. It allows any internet user to analyze data within our laboratory's database which represents the largest single center data set of microarrays in the United States. This data set encompasses many diseases, including arrays from humans with and animal models of asthma.^{25,26} Two other large publicly available databases are the NCBI GEO Web site (<http://www.ncbi.nih.gov/geo>) and the ArrayExpress site (<http://www.ebi.ac.uk/arrayexpress>).

The utility of microarray studies is readily apparent from a quick review of the literature^{27, 28} with the activation of the genes for arginase^{16,29–31} and amphiregulin, a profibrotic,³² in asthma identified by microarray studies. Microarrays can be especially effective when used as a hypothesis-generation tool along with novel software that incorporates the National Library of Medicine PubMed database of abstracts into microarray experimental analysis. Each gene transcript can be used much like another independent variable in these often longitudinal cohort studies. However, the importance of quality control and standard operating procedures are likely unsurpassed as small procedural errors can cause very significant changes in results.³³ Because of this, it is accepted practice to verify microarray findings with polymerase chain reaction of mRNA for select transcripts of interest. This technique has higher specificity.

FUTURE DIRECTIONS: IMPLICATIONS FOR ASTHMA EVALUATION AND MANAGEMENT

Polymorphisms in Asthma

Earlier in this article, we touched on some “asthma genes” that may contribute to an individual's risk for developing asthma and for incurring exacerbations. This has become an intense area of research. A search of the National Institutes of Health CRISP database showed 2004 studies with keywords of asthma and gene out of 7787 asthma studies in the prior 10 years.³⁴ Many of these are linkage analysis and association studies aimed at finding genes responsible in part for asthma risk. There are also quite a few microarray-based investigations targeted at determining novel pathways for asthma disease and exacerbation development.

A possibly more compelling direction for asthma research stems from the concept that SNPs may alter the function of receptors important in the treatment of asthma. In doing so, SNPs can contribute to changes in the response to therapeutics in several ways. First, they can alter the metabolism of a drug (ie, pharmacokinetic effect). For example, HIV-positive children with an SNP in the multidrug-resistance transporter gene (*MDR1*) had higher plasma levels of

nelfinavir in the highly active antiretroviral therapy regimen.³⁵ Second, SNPs can cause an adverse reaction in certain patients. A prime example of this is the HIV drug, abacavir, hypersensitivity. This reaction has been found to be 29 times more likely with a particular SNP in HLA B.^{36–42} Last, SNPs can alter the response to a drug at its target (ie, variant pharmacodynamic effects). This is most important in the concept of pharmacogenomics, where drug therapy is tailored toward the genotype of the patient. Although futuristic in some ways, pharmacogenomics is routinely practiced in oncology: cancers are genotyped and treated according to those results.

With regard to asthma, pharmacogenomics is very much in its infancy. That said, there is promise that, someday, practitioners may be able to tailor their asthma medication regimens to a patient's genotype. For example, SNPs and haplotypes in the β_2 -agonist receptor gene are suspected to explain some of the variability in therapeutic response seen among asthmatics.^{43–45} These findings bear further confirmation, but it is reasonable to suspect that there would be some culpability for genotype in this variability. In addition, SNPs are suspected to contribute to alterations in leukotriene metabolism^{46,47} and glucocorticoid responsiveness.^{48,49}

Despite what is likely to be a rapidly growing list of SNPs associated with asthma treatment efficacy, pharmacogenomics will require individuals' genotypes to be available to direct clinicians as to how to alter therapy for patients whose physiology varies because of these SNPs. The technology for high-throughput genotyping is readily available in many centers; however, and its cost is decreasing. For example, in our laboratory, we can genotype 4000 samples in 1 day at approximately US\$1 per sample (not including the initial cost of the sequencer). It is likely that chronically ill patients will be the first to benefit from this technology as they can be genotyped in their outpatient clinic and then that data would be available when they present acutely ill to the emergency department. The technology must improve to obtain real-time results (ie, in fewer than several hours) to impact not previously genotyped patients as they present to the emergency department. Even so, studies will need to be done to determine how to modify current treatments to most effectively treat patients with variant pharmacodynamics.

SUMMARY

In 2015, the 7-year-old chronic asthmatic mentioned at the beginning of the article has some genotype information known at the time of his presentation—it was collected at a pulmonary clinic visit the previous year after an ICU admission. Much like a history of a smoker at home, his parent may report that he has the “steroid gene” that you know means he carries a haplotype in the gene for one of his glucocorticoid receptors that makes him less sensitive to glucocorticoid treatment and thus requires higher doses and longer treatment. You adjust your therapy accordingly.

This patient scenario becomes closer to reality each year as genomic technologies rapidly mature. The role of environment in the asthma epidemic cannot be discounted—its role is clear and important. However, despite the long-held knowledge that genes are important in asthma as well, the study of genetics in asthma had been limited by the lack of a means by which to examine it. Relatively, little had changed since Sir William Osler wrote about asthma 100 years ago. Now, we are on the verge of significant change.

Acknowledgements

The authors thank Eric P. Hoffman, PhD, for his input during the preparation of this manuscript.

References

1. Brugge D, Vallarino J, Ascolillo L, et al. Comparison of multiple environmental factors for asthmatic children in public housing. *Indoor Air* 2003;13(1):18–27. [PubMed: 12608922]
2. Levy JI, Welker-Hood LK, Clougherty JE, et al. Lung function, asthma symptoms, and quality of life for children in public housing in Boston: a case-series analysis. *Environ Health* 2004;3(1):13. [PubMed: 15585065]
3. Barraza Villarreal A, Sanin Aguirre LH, Tellez Rojo MM, et al. Risk factors for asthma in school children from Ciudad Juarez, Chihuahua. *J Asthma* 2003;40(4):413–423. [PubMed: 12870837]
4. Lawson JA, Senthilselvan A. Asthma epidemiology: has the crisis passed? *Curr Opin Pulm Med* 2005;11(1):79–84. [PubMed: 15591893]
5. Mommers M, Gielkens-Sijstermans C, Swaen GM, et al. Trends in the prevalence of respiratory symptoms and treatment in Dutch children over a 12 year period: results of the fourth consecutive survey. *Thorax* 2005;60(2):97–99. [PubMed: 15681494]
6. Colilla S, Nicolae D, Pluzhnikov A, et al. Evidence for gene-environment interactions in a linkage study of asthma and smoking exposure. *J Allergy Clin Immunol* 2003;111(4):840–846. [PubMed: 12704367]
7. Sengler C, Lau S, Wahn U, et al. Interactions between genes and environmental factors in asthma and atopy: new developments. *Respir Res* 2002;3(1):7. [PubMed: 11806842]
8. Wright RJ, Cohen RT, Cohen S. The impact of stress on the development and expression of atopy. *Curr Opin Allergy Clin Immunol* 2005;5(1):23–29. [PubMed: 15643340]
9. Carroll W. Asthma genetics: pitfalls and triumphs. *Paediatr Respir Rev* 2005;6(1):68–74. [PubMed: 15698819]
10. Kurz T, Altmueller J, Strauch K, et al. A genome-wide screen on the genetics of atopy in a multiethnic European population reveals a major atopy locus on chromosome 3q21.3. *Allergy* 2005;60(2):192–199. [PubMed: 15647040]
11. Malerba G, Pignatti PF. A review of asthma genetics: gene expression studies and recent candidates. *J Appl Genet* 2005;46(1):93–104. [PubMed: 15741670]
12. Osler, W. *The Principles and Practice of Medicine*. New York: D. Appleton and Co; 1901.
13. NCBI GEO Web site. Bethesda, Md: NCBI; Available at: <http://www.ncbi.nlm.nih.gov/projects/SNP/>. [cited 2005 March 29]
14. Kruglyak L, Nickerson DA. Variation is the spice of life. *Nat Genet* 2001;27(3):234–236. [PubMed: 11242096]
15. Romualdi C, Balding D, Nasidze IS, et al. Patterns of human diversity, within and among continents, inferred from biallelic DNA polymorphisms. *Genome Res* 2002;12(4):602–612. [PubMed: 11932244]
16. Martinez, FD.; Kraft, M.; Lazarus, SC. 100th International Conference of the American Thoracic Society. Orlando, Fla: Joseph J Dennis; 2004. Emerging concepts for understanding asthma.
17. Hoffjan S, Ober C. Present status on the genetic studies of asthma. *Curr Opin Immunol* 2002;14(6):709–717. [PubMed: 12413520]
18. Hakonarson H, Halapi E. Genetic analyses in asthma: current concepts and future directions. *Am J Pharmacogenomics* 2002;2(3):155–166. [PubMed: 12383022]
19. Halapi E, Hakonarson H. Recent development in genomic and proteomic research for asthma. *Curr Opin Pulm Med* 2004;10(1):22–30. [PubMed: 14749602]
20. Kurz T, Ober C. The role of environmental tobacco smoke in genetic susceptibility to asthma. *Curr Opin Allergy Clin Immunol* 2004;4(5):335–339. [PubMed: 15349030]
21. Karjalainen J, Hulkkonen J, Nieminen MM, et al. Interleukin-10 gene promoter region polymorphism is associated with eosinophil count and circulating immunoglobulin E in adult asthma. *Clin Exp Allergy* 2003;33(1):78–83. [PubMed: 12534553]
22. Joos L, McIntyre L, Ruan J, et al. Association of IL-1beta and IL-1 receptor antagonist haplotypes with rate of decline in lung function in smokers. *Thorax* 2001;56(11):863–866. [PubMed: 11641511]
23. Joos L, He JQ, Shepherdson MB, et al. The role of matrix metalloproteinase polymorphisms in the rate of decline in lung function. *Hum Mol Genet* 2002;11(5):569–576. [PubMed: 11875051]

24. Brazma A, Hingamp P, Quackenbush J, et al. Minimum information about a microarray experiment (MIAME)—toward standards for microarray data. *Nat Genet* 2001;29(4):365–371. [PubMed: 11726920]
25. Seo J, Bakay M, Chen YW, et al. Interactively optimizing signal-to-noise ratios in expression profiling: project-specific algorithm selection and detection p-value weighting in Affymetrix microarrays. *Bioinformatics* 2004;20(16):2534–2544. [PubMed: 15117752]
26. Chen J, Zhao P, Massaro D, et al. The PEPR GeneChip data warehouse, and implementation of a dynamic time series query tool (SGQT) with graphical interface. *Nucleic Acids Res* 2004;32(database issue):D578–D581. [PubMed: 14681485]
27. Zou J, Young S, Zhu F, et al. Microarray profile of differentially expressed genes in a monkey model of allergic asthma. *Genome Biol* 2002;3(5):research0020.1–13. [PubMed: 12049661]
28. Erle DJ, Yang YH. Asthma investigators begin to reap the fruits of genomics. *Genome Biol* 2003;4(11):232. [PubMed: 14611649]
29. Zimmermann N, King NE, Laporte J, et al. Dissection of experimental asthma with DNA microarray analysis identifies arginase in asthma pathogenesis. *J Clin Invest* 2003;111(12):1863–1874. [PubMed: 12813022]
30. Vercelli D. Arginase: marker, effector, or candidate gene for asthma? *J Clin Invest* 2003;111(12):1815–1817. [PubMed: 12813015]
31. King NE, Rothenberg ME, Zimmermann N. Arginine in asthma and lung inflammation. *J Nutr* 2004;134(10 suppl):2830S–2836S. [PubMed: 15465795][discussion p2853S.]
32. Wang SW, Oh CK, Cho SH, et al. Amphiregulin expression in human mast cells and its effect on the primary human lung fibroblasts. *J Allergy Clin Immunol* 2005;115(2):287–294. [PubMed: 15696083]
33. Chen YW, Zhao P, Borup R, et al. Expression profiling in the muscular dystrophies: identification of novel aspects of molecular pathophysiology. *J Cell Biol* 2000;151(6):1321–1336. [PubMed: 11121445]
34. National Institutes of Health CRISP database. Bethesda, Md: National Institutes of Health; Available at: <http://crisp.cit.nih.gov/>. [cited 2005 April 5]
35. Saitoh A, Singh KK, Powell CA, et al. An MDR1-3435 variant is associated with higher plasma nelfinavir levels and more rapid virologic response in HIV-1 infected children. *AIDS* 2005;19(4):371–380. [PubMed: 15750390]
36. Hughes DA, Vilar FJ, Ward CC, et al. Cost-effectiveness analysis of HLA B*5701 genotyping in preventing abacavir hypersensitivity. *Pharmacogenetics* 2004;14(6):335–342. [PubMed: 15247625]
37. Watson ME, Pimenta JM, Spreen WR, et al. HLA-B*5701 and abacavir hypersensitivity. *Pharmacogenetics* 2004;14(11):783–784. [PubMed: 15564887][author reply p784.]
38. Martin AM, Nolan D, Gaudieri S, et al. Predisposition to abacavir hypersensitivity conferred by HLA-B*5701 and a haplotypic Hsp70-Hom variant. *Proc Natl Acad Sci U S A* 2004;101(12):4180–4185. [PubMed: 15024131]
39. Hughes AR, Mosteller M, Bansal AT, et al. Association of genetic variations in HLA-B region with hypersensitivity to abacavir in some, but not all, populations. *Pharmacogenomics* 2004;5(2):203–211. [PubMed: 15016610]
40. Mallal S, Nolan D, Witt C, et al. Association between presence of HLA-B*5701, HLA-DR7, and HLA-DQ3 and hypersensitivity to HIV-1 reverse-transcriptase inhibitor abacavir. *Lancet* 2002;359(9308):727–732. [PubMed: 11888582]
41. Hetherington S, Hughes AR, Mosteller M, et al. Genetic variations in HLA-B region and hypersensitivity reactions to abacavir. *Lancet* 2002;359(9312):1121–1122. [PubMed: 11943262]
42. Flexner C. HLA B57 and abacavir hypersensitivity. *Hopkins HIV Rep* 2002;14(3):5. [PubMed: 12206099]
43. Westland R, van Veen A, Jansen HM, et al. Limited impact of multiple 5' single-nucleotide polymorphisms on the transcriptional control of the human beta 2-adrenoceptor gene. *Immunogenetics* 2004;56(9):625–630. [PubMed: 15578262]
44. Yoshida N, Nishimaki Y, Sugiyama M, et al. SNP genotyping in the beta(2)-adrenergic receptor by electronic microchip assay, DHPLC, and direct sequencing. *J Hum Genet* 2002;47(9):500–503. [PubMed: 12202992]

45. Yoshida N, Sugiyama M, Tanoue A, et al. Determination of a novel haplotype of beta2-adrenergic receptor in the Japanese population by the combination of the electronic microchip assay using the NanoChip system with allele-specific PCR. *Drug Metab Pharmacokinet* 2002;17(6):532–539. [PubMed: 15618708]
46. Eilertsen KE, Olsen JO, Brox J, et al. Association of the -159 C→T polymorphism in the CD14 promoter with variations in serum lipoproteins in healthy subjects. *Blood Coagul Fibrinolysis* 2003;14(7):663–670. [PubMed: 14517492]
47. Sanak M, Pierzchalska M, Bazan-Socha S, et al. Enhanced expression of the leukotriene C(4) synthase due to overactive transcription of an allelic variant associated with aspirin-intolerant asthma. *Am J Respir Cell Mol Biol* 2000;23(3):290–296. [PubMed: 10970818]
48. Uhart M, McCaul ME, Oswald LM, et al. *GABRA6* gene polymorphism and an attenuated stress response. *Mol Psychiatry* 2004;9(11):998–1006. [PubMed: 15197399]
49. Koyano S, Saito Y, Sai K, et al. Novel genetic polymorphisms in the *NR3C1* (glucocorticoid receptor) gene in a Japanese population. *Drug Metab Pharmacokinet* 2005;20(1):79–84. [PubMed: 15770078]

FIGURE 1.

Transcription and translation. DNA is used as a template by RNA polymerase enzymes to form mRNA from nucleotides. This single-stranded, easily degraded molecule is transported to the ribosomes where it is used as a template for the assembly of proteins from amino acid components.

FIGURE 2.

The process for determination of mRNA relative abundance in tissue. Tissue mRNA is reverse transcribed into single- and then double-stranded complementary DNA. This complementary DNA is then transcribed using an abundance of fluorescent-labeled (B) nucleotides to form fluorescent complementary RNA which is then enzymatically fragmented. These oligonucleotides are allowed to hybridize to a “chip” onto which corresponding oligonucleotide probe sets for the entire genome have been bound. The relative level of fluorescence for each probe set allows the computer software to determine the relative mRNA abundance in the original tissue.