

## REVIEW ARTICLE

## GENOMIC MEDICINE

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## Genomics as a Probe for Disease Biology

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**A**LTHOUGH OUR UNDERSTANDING OF PATHOLOGY HAS GROWN RAPIDLY in recent decades, the underlying mechanisms of many diseases remain obscure. Genomic research offers a new opportunity for determining how diseases occur, by taking advantage of experiments of nature and a growing array of sophisticated research tools to identify the molecular abnormalities underlying disease processes.<sup>1</sup> In this review I examine examples in which genomic research has improved our understanding of molecular pathobiology and consider its potential for contributing to the study of common complex diseases.

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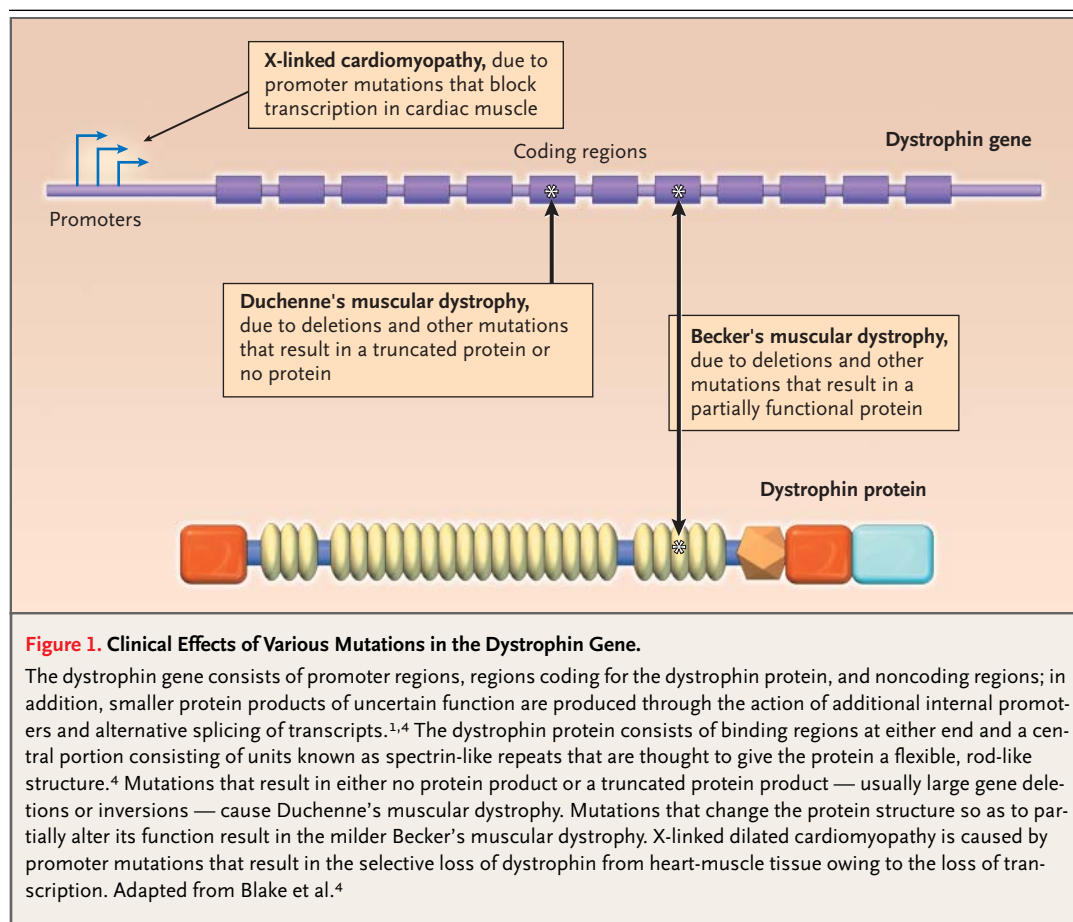
## EFFECT OF MUTATIONS ON THE SEVERITY OF HEMOPHILIA A

Before the advent of therapy for hemophilia A, some affected patients had only moderate bleeding problems, lived to adulthood, and led relatively normal lives in the absence of trauma or surgery.<sup>2</sup> Others had severe, spontaneous bleeding beginning in early childhood and rarely survived to adulthood; still others had disease of intermediate clinical severity. The main cause of this variation is the different mutations of the hemophilia A gene that cause hemophilia. When a mutation results in the complete loss of factor VIII protein — usually because a large gene deletion or genetic inversion results in the failure of gene transcription — severe hemophilia occurs.<sup>2</sup> The absence of endogenous factor VIII also increases the likelihood of an immune response when factor VIII–replacement therapy is used.<sup>3</sup> This immune response is a severe complication because it can prevent effective treatment. In contrast, other mutations, such as those in which there is a small change in the DNA sequence of the gene, lead to amino acid substitutions in the factor VIII protein. Depending on the nature of the substitution and its effect on the function of factor VIII, these mutations cause mild-to-moderate disease.

This example demonstrates that there is often a logical relation between a person's DNA sequence (genotype) and health outcome (phenotype). The functional effect of the genotype is the key factor, providing a molecular explanation for the severity of a given disease.

## RECLASSIFICATION OF THE DYSTROPHINOPATHIES THROUGH GENOMIC UNDERSTANDING

Duchenne's muscular dystrophy is caused by mutations in the dystrophin gene — usually deletions or gene inversions — that produce total or near-total loss of the dystrophin protein from skeletal muscle, resulting in early and progressive loss of muscle function (Fig. 1).<sup>5</sup> Mutations in the dystrophin gene that cause less severe deficits in the final protein product result in Becker's muscular dystrophy, a milder disease that was historically considered a separate clinical entity. This disorder differs from Duchenne's muscular dystrophy in its later onset and milder course.<sup>5</sup>



When genomic research showed that these two clinically distinct disorders involved the same gene, a family of clinical disorders known as dystrophinopathies was identified.<sup>4</sup> A third dystrophinopathy, X-linked dilated cardiomyopathy, was subsequently discovered. This disorder is caused by specific mutations in the dystrophin gene that lead to the selective loss of dystrophin from cardiac muscle, while dystrophin levels in skeletal muscles remain normal or nearly normal.<sup>4,6</sup> These mutations appear to affect one of the promoter regions of the gene, resulting in the selective loss of gene transcription in cardiac tissue (Fig. 1).<sup>4,6</sup> Discovery of the gene coding for dystrophin thus provided the means to understand a molecular relation among three seemingly different clinical disorders. As with hemophilia A, the relation between the genotype and the clinical outcome is the result of the functional effect of different mutations on dystrophin.

Another genetic disorder, hereditary hemorrhagic telangiectasia,<sup>7</sup> provides an interesting contrast.

This disorder (also known as Osler–Weber–Rendu disease) causes vascular dysplasia, resulting in epistaxis, hemoptysis, and gastrointestinal bleeding. It is inherited as an autosomal dominant disorder and was assumed to be due to mutations in a single gene, but molecular studies revealed the involvement of two genes, one encoding activin-receptor-like kinase 1 (ALK1)<sup>8</sup> and one encoding endoglin.<sup>9</sup> Thus, the discovery of the genetic causes in this case revealed an unexpected complexity. Molecular studies also provided an explanation for the similar clinical outcome of mutations in two different genes: both protein products appear to function in the same or related biologic pathways.<sup>8,9</sup>

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VARIABLE EFFECT OF THE SAME  
GENOTYPE ON HEALTH OUTCOMES  
IN CYSTIC FIBROSIS

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Although the clinical effect of genetic mutations can often be predicted on the basis of their functional

effect, the relation between the genotype and phenotype is sometimes less direct, as illustrated by cystic fibrosis. Cystic fibrosis results from mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene; different mutations in the gene have various effects on the function of CFTR protein.<sup>10,11</sup> More severe mutations are associated with a loss of function, through defective synthesis, defective maturation, or blocked activation of the CFTR protein. Other mutations result in only partial loss of CFTR function, and as expected, these mutations are often associated with less severe disease,<sup>10,11</sup> including a later onset of symptoms, pancreatic sufficiency, and sometimes milder pulmonary disease.<sup>3,12,13</sup>

However, the correlation between genotype and phenotype in cystic fibrosis is imprecise. In particular, the severity of pulmonary disease cannot be predicted for most CFTR genotypes, including the most common one —  $\Delta F508/\Delta F508$ <sup>11</sup> — since both the age at onset of pulmonary symptoms and the rate of decline in pulmonary function vary.<sup>14-16</sup> For example, in some patients with the  $\Delta F508/\Delta F508$  genotype, chronic respiratory symptoms do not develop until adolescence or adulthood and pulmonary function in young adulthood can range from highly compromised to normal.<sup>11,15,16</sup> This discrepancy is important, because pulmonary complications are typically the most serious and life-threatening manifestations of cystic fibrosis.

Both genetic and nongenetic factors appear to modify the effect of the cystic fibrosis genotype.<sup>11</sup> A Danish study found that certain genetic variants of mannose-binding lectin, a protein that functions in innate immune responses, were associated with greater loss of lung function in patients with cystic fibrosis.<sup>17</sup> Interestingly, this adverse effect was limited to patients with chronic *Pseudomonas aeruginosa* infection, indicating an interaction between the gene variant and the environment. Other studies, some of which used animal models of cystic fibrosis, provide additional evidence of the existence of genetic modifiers of the clinical effect of CFTR genotypes.<sup>11,18-20</sup> Some modifiers appear to be organ-specific; for example, a gene locus has been identified that influences whether an infant with cystic fibrosis will have neonatal meconium ileus, but it does not appear to influence the severity of lung disease.<sup>21</sup> Nongenetic modifiers of the cystic fibrosis phenotype, such as exposure to environmental tobacco smoke as a factor in the poor outcome of the disease<sup>22</sup> or exposure to respiratory pathogens as a

contributor to the progression of lung disease, have been described.<sup>10,11,23</sup> The existence of environmental modifiers is also suggested by studies demonstrating a correlation between life expectancy and health insurance status<sup>24</sup> or socioeconomic status.<sup>25</sup> These findings could reflect the modifying effect of factors such as nutritional status or access to antibiotic treatment or could represent the effect of other environmental modifiers that have yet to be described.

Further evidence of the complexity of the genetics of cystic fibrosis comes from data on other phenotypes associated with CFTR mutations. Some mutations cause male infertility through the congenital absence of the vas deferens, without accompanying lung disease,<sup>26</sup> and others cause idiopathic chronic pancreatitis and mild, late-onset pulmonary disease.<sup>11,27</sup> Limited data suggest that CFTR mutations may also contribute to some cases of chronic sinusitis and allergic bronchopulmonary aspergillosis.<sup>27</sup> As we learn more about the function of CFTR and the effect of other genetic and nongenetic factors on this protein, we will most likely increase our understanding of the biology of both cystic fibrosis and related disorders.

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#### GENETIC VARIANTS AS A COMMON PHENOMENON

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Variants in many different genes form the basis for the genetic contribution to disease across the spectrum, from rare disorders such as cystic fibrosis to common complex disorders such as cancer and heart disease. Genetic variants occur because new mutations arise at a low but continual rate in human tissues. Mutations that arise in germ-line tissues can subsequently be inherited, increasing the genetic variation in the population. The recently completed draft sequence of the human genome includes a catalog of 1.4 million single-nucleotide polymorphisms — sites where variations occur in the bases that form the building blocks of the DNA sequence.<sup>28</sup> Most DNA-sequence variations occur in noncoding regions of the genome — that is, in regions that do not code for protein products. Changes that occur in coding regions, however, can affect the function or efficiency of the protein that a gene encodes.

These differences can have physiological effects that are clinically important, such as causing differences in the response to drugs or environmental exposures or differences in susceptibility or the pre-

disposition to diseases. For example, many of the enzymes responsible for drug metabolism occur in variant forms, leading to differences between people in the efficacy of a drug and the risk of adverse effects.<sup>29,30</sup> Similarly, many genetic variants that contribute to the risk of common diseases such as cancer and heart disease have been identified.<sup>31</sup> Unlike the gene changes that cause genetic disease, which tend to be rare and result in clinically significant loss of function, most common genetic variants cause relatively small changes in function.

Genetic changes may be adaptive in certain circumstances and harmful in others. An often-cited example is the association of sickle cell trait — a mutation in the hemoglobin A gene — with resistance to malaria.<sup>32</sup> The selective advantage conferred by this resistance accounts for the high prevalence of sickle trait in populations originating from regions where malaria is endemic. However, the presence of two copies of the sickle cell trait results in sickle cell anemia, a disease associated with multiple complications and premature mortality. In this case, genetics offered insights into disease biology that would not otherwise have been attainable. The Human Genome Project is likely to document many instances in which the effects of genetic variation are dependent on the context. As an example, a variant in the interleukin-1-receptor agonist, a protein that inhibits the inflammatory response, is associated with an increased risk of certain autoimmune diseases, but it may reduce the morbidity of some infections.<sup>33</sup>

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ASTHMA AS AN EXAMPLE  
OF THE GENETICS  
OF COMMON COMPLEX DISEASES

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Both genetic and environmental risk factors have an important role in most common diseases.<sup>31</sup> The same strategies developed for the study of genetic diseases can be applied to the study of common diseases, but the task is more difficult because complex patterns of gene–gene and gene–environment interactions must be evaluated. Asthma provides an illustration of this challenge.

Epidemiologic studies provide evidence that multiple genetic and environmental factors contribute to the causation of asthma, a clinical condition that is best viewed as a cluster of related disorders.<sup>34</sup> Patients with asthma vary with respect to the age at onset, course, sensitivity to specific environmental precipitants, and response to medications, and the

relative contribution of genetic and nongenetic factors may also vary considerably among patients. Furthermore, the prevalence of asthma has risen dramatically in the past two decades, indicating that environmental risk factors have a key role.<sup>35</sup> Control of environmental risk factors and improved treatment are the primary public health strategies for the prevention of asthma.

Nevertheless, genetic factors contribute substantially to the risk of asthma.<sup>34,36–38</sup> How, in this context, might the study of the genetics of asthma contribute to better health care outcomes? There are several possibilities. A classification of asthma based on genetics might provide a more accurate means of defining clinical subtypes that benefited from specific treatments. Genetic classification might also provide improved prognostic information, including the identification of patients who are at highest risk for severe or life-threatening episodes of asthma. A better understanding of the molecular processes involved in the different pathways of asthma is also likely to lead to a more detailed understanding of the pathophysiology of the disease. This effort could lead to a more precise definition of the environmental modifications most likely to reduce the risk of asthma. It could also lead to improved drug treatment through genetic testing to predict a patient's responses to a drug or through the development of new drug therapies.<sup>36,38</sup>

Studies to identify genes associated with asthma use mapping techniques to pinpoint gene loci linked to asthma<sup>1</sup> and physiological studies to identify genes that are likely to affect the disease process. Both approaches have been productive. Mapping techniques have identified several genes associated with asthma.<sup>37,38</sup> One of these has yielded information about a metalloproteinase, ADAM-33, that may have a role in inflammatory responses or smooth-muscle hypertrophy and hyperreactivity.<sup>39,40</sup> Physiological studies have led to the characterization of genetic variants associated with asthma or atopic airway inflammation in several biologic pathways potentially related to asthma — for example, the beta-adrenergic receptor,<sup>41</sup> cytokines associated with the secretion of immunoglobulin E and airway inflammation,<sup>42</sup> and a transferase presumed to be involved in the detoxification of inhaled irritants.<sup>43</sup> One study reported a gene–environment interaction in which the effect of smoking on the risk of asthma was increased by a specific beta-adrenergic-receptor genotype.<sup>44</sup>

This research is still in the early stages and faces

a number of technical problems that will also apply to the study of other common diseases. These include the need for standardized definitions of asthma phenotypes and intermediate biologic measures associated with the risk of asthma,<sup>45-47</sup> well-defined populations in unbiased studies with sufficient power to detect small effects,<sup>48-50</sup> and the concurrent measurement of both environmental and genetic risk factors.<sup>51</sup> Finally, any reported association between a genetic variant and disease risk cannot be considered established until the results of the study have been replicated.<sup>52,53</sup>

Determining associations between genes and diseases is just the first step in the translation of genomic research into clinical insights. This effort will require increasing attention to the study of the functions of proteins, or "proteomics,"<sup>54</sup> including the characterization of proteins identified as a result of genomic research. Of the estimated 30,000 to 35,000 genes in the human genome, approximately half code for unknown proteins.<sup>28</sup> With use of the genetic techniques that are now available, these proteins, their functions, and their interactions will be increasingly identifiable.<sup>55</sup> Analysis of the interaction between genetic and environmental effects in relevant biologic pathways, some (perhaps many) of which remain to be discovered, will form an im-

portant part of the study of common diseases such as asthma. As methods to achieve this goal are developed, they will provide a more complete and detailed picture of disease processes than has ever previously been possible.<sup>56</sup>

CONCLUSIONS

The study of gene mutations has provided a new model of pathophysiology in which the molecular causes of disease are illuminated by genetics. Evidence is now emerging of the complex interactions between genes and between genes and the environment in the causation of many diseases, and the study of these interactions represents the next important step in genomic research. Efforts to understand the molecular mechanisms that underlie common complex diseases will build on insights and strategies developed in the study of single-gene diseases. However, the scope of the analysis is far greater and will require continuing efforts to develop and improve molecular and informatic tools that allow the simultaneous analysis of many genetic variants and environmental risk factors. The success of genomic research to date<sup>28</sup> suggests that this ambitious research enterprise will also ultimately succeed.

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