# GENOMICS AND NEUROLOGY

*The Fourth Pair of Articles in a Series about the Significance of Genetic Science for Catholic Health Care*

## A MEDICAL VIEW

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We have entered "the post-genome era" of science and medicine. A wealth of information has been uncovered in the past decade. Most notably, the 13-year effort to sequence the human genome was completed. The completion of the sequencing is a monumental landmark that has modified drastically our original approximation of the number of genes in the human genome. At the start of the project, we estimated that 100,000 genes were present on the 23 pairs of chromosomes. The most recent estimate reported by the International Human Genome Sequencing Consortium is that there are 20,000 to 25,000 protein-coding genes. Several sources agree that more than 50 percent of these genes are encoded in the brain. This makes a discussion about genomics and neurology a daunting task. Considering the many types of neurological disorders, I am narrowing the scope of this discussion to a few conditions for which interesting genomic research has been occurring.

Genomic neurology obviously comprises two disciplines: neurology and genetics/genomics. About half a century ago, the field of neurology was drastically different than today (and the field of genomics was not even born!). For most disorders of the brain and nervous system, there were no interventions available. Neurologists could do little more than describe the condition from which a patient was suffering, and possibly prognosticate.[1] Physicians believed that nerve cells could not regenerate, and that the brain was "hard wired." Neurology made significant advances when direct DNA testing for neurological disorders became available. Now we are in a new era of medicine, attempting to discover the mechanisms of disease. We believe that new nerve cells *can* form and function and that the circuitry of the brain has plasticity, and that it *is* modified by an individuals' environment. This plasticity may be the key to reversing neural illness and injury in the future.

In the field of medical genetics, concepts and practice have also changed. We once considered most genetic conditions "Mendelian," meaning that traits are inherited in a pattern that is either dominant (one altered gene causes a change or disease) or recessive (two of the same altered genes cause a change or disease). Medical practice often consisted of describing a given condition, taking a family history in an attempt to ascertain the inheritance pattern, and predicting who else in a kindred might be at risk. Prevention of disease then involved reproductive counseling, rather than treatment.

We now are well aware that many of the most common genetic conditions are not Mendelian, but, rather, complex traits. We hypothesize that many neurological conditions are "multifactorial"—they involve genes that modify other genes and genes that are influenced by environmental factors. Some gene alterations may not confer absolute disease on an individual, but may alter a person's susceptibility to disease or be involved in the mechanism of disease. Determining the interactions of all the genes and how they may lead to disease—this is the core of genomics.

### NEW TECHNOLOGIES, NEW KNOWLEDGE

Many conditions for which neurologists see patients cannot yet be adequately treated.

Moreover, we do not fully understand the pathology of many of the neurological/neuromuscular diagnoses. We have only just begun to be able to offer genetic testing to confirm a molecular diagnosis for several of the more common neurological conditions.

Undoubtedly, molecular diagnosis has led us to a better understanding of the pathology of many conditions. It has even led to the definition of new diseases and of disease mechanisms, which are today applied to diseases that were once classified as nongenetic. However, we have a long road ahead before we arrive at a full understanding of the complex neurological diseases and how to test for them. In predictive genomic testing based on risk factors, one challenge is that each risk factor is just one of several factors likely contributing to the more common diseases. Having the risk factor does not necessarily indicate that one will develop a given condition.

Researchers have found that a large proportion of the neurally expressed genes are highly conserved among such organisms as drosophila (a small, two-winged fly) and nematodes (a worm species). This allows us to use animal models to identify the genes and proteins involved in neurological pathogenesis, and try to move toward therapeutic discoveries. We have learned that many genes found in humans have counterparts in lower organisms. For example, presenilin 1, presenilin 2, and amyloid beta-precursor protein, which play a role in familial early-onset Alzheimer's disease (AD), have been found and studied in fruit flies. Deletion of the drosophila beta-amyloid precursor protein-like gene leads to behavioral defects that can be partially restored by transgenic expression of the human amyloid precursor protein.[2]

By studying drosophila and other organisms, we have been able to better understand the molecular mechanisms involved in neurodegeneration. We have discovered that the complexity and diversity of the human genome (as against that of invertebrates) is related not to the number of genes, but, rather, to the increase in regulator genes like those coding for transcription factors (i.e., genes that help control other genes). This is especially true in the brain, where a high percentage of genes are preferentially or exclusively expressed.[3]

Scientists are developing more sophisticated technologies and data analysis methods to further genomics research. The Human Genome Project (HGP) has stimulated the development of technologies for high-throughput genome analysis, such as microarray technology and the fluorescent sequencer. These advances enabled us to generate a detailed catalogue of genes that constitute our genome. We are in the process of deciphering the functions of each of these genes. For instance, we now have the ability to analyze the transcription of up to 10,000 genes by doing a single experiment. This makes it possible for researchers to investigate the differences between various tissue types and to explore the alterations in that expression pattern during disease.

For the past few years, researchers have been able to utilize technology to complete systematic scans (called "linkage scans") of the genome to try to find genes associated with genetic risk. Classical linkage analysis traditionally involves looking for regions in the genome that are usually transmitted through a family in a way that parallels the transmission of a given trait.[4] Getting desired results requires studying large family trees over long periods of time. Recent progress in deciphering genes in the genome via high-volume technology has made it possible to search the genome for risk-associated variants. This technology, called "genomewide scanning," involves thousands of genetic markers and subjects.[5] It allows one to search for linkage throughout the genome by utilizing a set of genetic landmarks spaced evenly over all chromosomes. This is an important method in the study of complex diseases.

Animal studies have added to our knowledge of neurodegeneration.

## Recent Findings

Whole genome scans currently are being performed for many conditions, and we enthusiastically await the results of these studies. In the meantime, I will provide an overview of some of the latest research discoveries in genomic neurology.

**Multiple Sclerosis** Multiple sclerosis (MS) is a condition noted for its relatively high frequency, chronic course, and tendency to manifest itself in young adults. MS results from the destruction of the protective sheath that insulates nerve cells (myelin). Sufferers experience episodes of focal

disturbance of the optic nerves, spinal cord, and brain, which remit and recur over many years.[6] The disease's unpredictable course makes it a condition for which many patients and physicians would like to see effective treatment developed.

The migraine headache is a condition that has long puzzled neurologists.

Before treatment is a reality, however, we need to better understand the condition's etiology.

The many studies done of MS point to a multifactorial etiology. The disease's familial clustering and the increased risk observed in siblings of affected individuals and monozygotic twins (as compared to the general population) implicate a genetic component of this condition. Fifteen percent of MS patients have an affected relative.[7] Other studies have looked at migration, month of birth, rural versus urban lifestyle, an increase in prevalence with an increase in latitude, and other factors, all of which suggest that the pathogenesis of MS is influenced by the environment.

In an attempt to unravel the disease's complexity, a recent study by the International Multiple Sclerosis Genetics Consortium (IMSGC) was conducted. This study has demonstrated that new scientific tools (a result of the HGP) can achieve a significant increase in power, as compared to previous methods of identifying susceptibility genes. By using linkage analysis, the IMSGC scientists found significant linkage in the major histocompatability complex (MHC) at 6p21 and suggestive linkage on 17q23 and 5q33. More than 10 such studies have been published about MS; however, none of these screens demonstrated linkage with genomewide significance.

The IMSGC study utilized more extensively engineered, higher-density microsatellite markers and high-density SNP-based mapping sets (Illumina BedArray linkage mapping panel and the Affymetrix GeneChip array). This study showed that nonsignificant statistical linkage was seen outside the MHC region, which could indicate that multiple effects of several susceptibility genes may contribute to MS. It seems reasonable to suggest that patients developing MS due to susceptibility genes at particular chromosomal loci might show symptomatic differences from those who develop MS due to a different set of susceptibility genes.[8] This could explain the difference in disease characteristics between individuals affected with MS.

**Alzheimer's Disease** Research on the genomic characterization of AD has implicated more than 180 genes distributed across the human genome as being potentially involved in the disease's pathogenesis.[9] We have found that, in rare families, AD is inherited as a Mendelian genetic condition. More commonly, older individuals possess several genes that in sum increase their risk of disease.

Evidence suggests that the genetic component in AD differs from other forms of dementia. In the past decade, we have significantly increased our understanding of this condition. The current hypothesis of disease mechanism is that a fragment of protein called AB-42 accumulates in the brain and is toxic to nerve cells.[10] Treatment approaches include trying to prevent the build up of this protein. As we learn more about all the susceptibility genes that likely work together to cause AD, we hope to be able to identify the at-risk population so that we can introduce lifestyle modifications, medications, or genetic modifications to reduce the condition's impact.

**Migraine Headaches** Another debilitating condition whose physiological basis has puzzled neurologists is the migraine headache. This complex disorder affects as many as 24 million people in the United States. Estimates suggest that from 18 to 20 percent of women and from 6 to 9 percent of men suffer at least one migraine per year. Susceptibility to a migraine may be inherited, and likely requires environmental stimuli to become manifest. A child whose parent experiences migraines has up to a 50 percent chance of developing migraine. The risk increases to 70 percent if both parents experience it. While familial migraine is common, we know that many cases have no apparent familial basis.

New genomic technology is allowing us to better characterize which genes may confer susceptibility to migraine. A recent genomewide linkage analysis involving a large cohort of participants has shown intriguing data.[11] Quantitative-trait linkage analysis produced evidence of statistically significant linkage on chromosome 5q21, and suggestive linkage on chromosomes 8, 10, and 13. Previously reported susceptibility loci on chromosomes 6p12 and 1q21 were replicated.[12] The authors used a technique called "latent class analysis" to find subtypes of related symptoms

from multivariate categorical data.

Having performed linkage analyses on the individual migraine symptoms for six chromosomes that demonstrated linkage, the authors reported some very interesting findings. For example, the susceptibility locus on chromosome 1 is most associated with phonophobia. The chromosome 6 locus is mostly associated with photophobia, while the chromosome 8 locus is associated with nausea and/or vomiting and moderate to severe headache. These results display the heterogeneous nature—and phenotypic and genomic complexity—of migraine. Research such as this may enable us to better diagnose migraines and understand physiologic and environmental influences.

**Autism** Many studies have been done to explore the neurodevelopmental disorder that is affecting as many as 1 in 300 children in metropolitan areas of the United States.[13] Of course, I am referring to autism, a spectrum disorder that usually manifests itself in the first three years of life. It is characterized by impairments in social interaction, communication, and possibly by the presence of repetitive patterns of interest or behavior.[14] This condition has a strong genetic component, as indicated by twin studies, and by a significantly greater risk to siblings than to the general population. More than 10 genomewide autism screens have been performed, and results indicate potential susceptibility genes spread across the entire genome. Autism may involve anywhere from 3 to 100 genes, depending on which study one consults. It is the epitome of a complex genomic condition.

**Neuromuscular Disorders** If we move on to the branch of neurology concerned with neuromuscular disorders, we can see how genomics may provide a better understanding of these conditions, too.

The last decade has witnessed remarkable progress in finding the causes of inherited muscle disorders. There are numerous genetic muscle diseases, all of which may affect each individual differently. More than 40 different genes have been identified that contain the instructions for normal muscle development. An error in any one of these may result in muscle disease.

We must understand the major pathways involved in homeostasis of the healthy human organism in order to understand how those pathways are altered in a state of illness. The identification of each gene that harbors a high-risk variant will point toward a pathway involved in illness. This notion has lead researchers to actively study neuromuscular-related genes and the different alternations within them. We still do not understand the pathogenesis of muscle cell degeneration, but we do know that there is a consistent variation in the way genes that cause muscular dystrophy affect different skeletal muscle groups.

With the availability of microarrays, we can now attempt to make the link between genes and anatomy. One can study the genes that are expressed in different regions of the brain, or in different muscles, and can identify the genes that participate in different functions specific to each anatomical region. This will help researchers to better comprehend region-specific neurological or muscular functions and facilitate the mapping of susceptibility genes for neurological conditions.

In an attempt to study muscle gene expression, researchers studied control muscle specimens to better understand the phenotypic difference seen in muscle disease.[15] Previous studies in mice have shown that differences in gene expression exist among muscle types. To determine whether variations in gene expression contribute to visible symptom patterns in the human muscular dystrophies, the authors of this study performed ribonucleic acid (RNA) profiling on four skeletal muscle groups unaffected by neuromuscular disease. RNA targets were prepared from all the samples and hybridized to Affymetrix GeneChips. Hierarchical clustering analysis was used to graphically display correlations in gene expression levels, along with other sophisticated analysis techniques.

The dominant factors affecting stratification in this study were found to be individuality, muscle type, and age. It is possible that environmental factors such as physical fitness and general health may have contributed to these factors. Despite significant interindividual variations, the analysis revealed molecular differences among human skeletal muscle types that are similar in basic histology.[16] This study forms the basis of an anatomical map of human skeletal muscle gene expression that, in the future, may help explain why generalized genetic insults lead to consistent patterns of weakness that affect some muscle types more than others.[17]

**Facioscapulohumeral Muscular Dystrophy (FSHD)** This is a neuromuscular condition whose genomic

nature has yet to be fully understood. The third most common form of muscular dystrophy, FSHD affects muscles of the face, shoulders, and upper arms, causing progressive weakness. Symptoms usually appear in the teenage years. The molecular lesion is a deletion within a large, complex DNA tandem array termed D4Z4 on chromosome 4q. Research has identified that the end of chromosome 4q has two variants: either 4qA or 4qB. These alleles seem to be present at roughly equal frequencies in the general population, but FSHD is associated with the 4qA allele.[18] This suggests a functional difference between the 4qA and 4qB variants, one of which may predispose to deletions in D4Z4 or in the pathological consequence of the deletion. Many researchers and patients anxiously await a more comprehensive definition of the pathology of this condition.

Medical scientists hope to better understand the genetic influences that govern our responses to therapies, so that we can tailor treatment plans to each individual.

### Looking into the Future

The genomic research findings discussed above have so far not been implemented in clinical use. Clinical utility may be derived in the near future for treatment of a specific type of brain tumor. Medulloblastomas are the most common malignant brain tumors in children, accounting for from 15 to 20 percent of pediatric brain tumors. This tumor is located in the cerebellum, which controls balance and other complex motor function. Long-term survival rates range from 60 to 85 percent, but cognitive deficits and other side effects of therapy are common in children who survive. Physicians are therefore trying to differentiate high- from low-risk patients in order to tailor therapy to the degree of biological aggressiveness of the tumor.

The most accurate outcome predictions to date have been obtained through microarray gene expression profiling.[19] The researchers involved conducted a study to see whether gene expression-based outcome predictions can be improved by combining clinical (i.e., age at diagnosis and size of tumor) and molecular analysis for disease stratification.[20] They tested whether gene expression predicts outcome independently (and whether clinical parameters add significantly to its accuracy). The researchers concluded that gene expression profiling does provide prognostic information that cannot be derived from histological or clinical criteria. If other researchers are able to confirm these results in larger prospective studies, molecular profiling may become the new standard for risk classification in future clinical trials.

Inevitably, the analysis of the human genomic sequence and interactions with the environment will be the focal point of research for years to come. For many of the complex neurological conditions, it seems plausible that susceptibility will be found to consist of a spectrum of relatively common susceptibility genes exerting only minimal effects, as well as more rare risk genes with more significant effects. Epistasis (gene-gene interaction) has been widely accepted as an important contributor to the complexity of mapping complex disease. The rapidly changing technologies stemming from genomics will continue to evolve and likely lead to the identification and characterization of all the functioning neuronal genes. This achievement, combined with technologies such as DNA microarrays and polymorphisms, will allow individual genetic analysis at a level much more precise than is currently possible. We may be able to use such information to learn which genetic mechanisms protect us from disease—and which increase our risk of it.

We medical scientists hope to better understand the genetic influences that govern our responses to therapies, so that we can tailor treatment plans to each individual. Since each individual has a different susceptibility to disease, based on his or her genetic makeup, the risk of developing adverse reactions to drugs also is individual. It is likely that the next step in medical treatment is to develop preventive measures and treatment plans specific to one's genetic constitution (the so-called "designer drugs").

In the next quarter century, we may see the manipulation of disease as an apparent theme. With luck, we will be able to prevent some neurological illnesses or alter their progression. For some conditions, we may be able to give patients hope of recovery. We may be able to modify existing genes, or introduce new ones, to make

proteins that the brain needs for improved functioning. And we may reach a point in time when stem cells are a form of successful treatment.

Stem cells are primitive cells with multipotential properties. Originally taken from embryonic tissue, they are now obtained from a variety of mature tissues, such as muscle or brain.[21] Many individuals see stem cells as a potential treatment for a wide variety of central nervous system disorders, including AD, Parkinson's disease, spinal cord injury, and stroke.[22] Inevitably, the future will entail more stem cell research and, potentially, therapies in which stem cells and progenitor cells will modify brain disease.

It is reasonable to say that, except for accidental injuries, essentially every human condition (functional or dysfunctional) has a hereditary component. The future will open volumes of information on the genetics of common illness. As a result, a wide variety of new genetic tests will become available to patients.

Who will be responsible for meeting all of society's genomic "wants and needs"? Genomic medicine will require physicians to become savvy at understanding, interpreting, and relaying genetic testing information. Physicians will be confronted with understanding patients' genomic makeups and their responsiveness to drugs in mainstream medicine. More genetic counselors will be needed to meet the demand for genetic information. When one considers the emotional ramifications of a genetic disease on a family—and especially the fact that preclinical determination of genetic disease can cause significant psychological burden—one sees that genetic counseling is vital.

The accumulation of genomic information produced by the new technology is outpacing the medical community's ability to implement this information clinically. In addition, social, legal, and ethical norms have not been developed to deal with it. As yet, society has said nothing about who should or should not be tested for genomic susceptibility.

If testing is offered for a susceptibility gene that we know is responsible for a particular condition—but a condition that we cannot yet treat—should the medical community provide this information to the patient? Will it cause more harm than good? These are questions that will need to be addressed and answered in the very near future. ■

## NOTES


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