# Applying advances in neurogenetics to medical practice

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The investigation of rare neurogenetic diseases is an example of how a translational science approach may lead to the delineation of complex genetic and biochemical pathways. Thisprocess comprises several intellectual stages. The first step involves the astute identification and clinical description of the unique phenotype, which may lead to obvious pathways or may reveal novel or unexpected mechanisms. As similar patients are identified, the establishment of databases detailing the clinical phenotype may serve to provide clues as to the genetic and biochemical characterization, and identification of the genetic mutation based on patient samples and animal or cellular models. Lastly, attempts to develop and apply therapies based on what has been learned about the biochemical and molecular bases of the disease enables intervention on the individual patient level. Several stages of discovery may overlap or be investigated simultaneously. As examples, this review discusses how this process of investigation has enabled progress in the delineation of several genetic and neurogenetic disorders, including Progeria syndrome, neurodegenerative diseases, muscular dystrophy, Rett syndrome and neurotransmitter disorders. This review attempts to summarize the transition from the bedside-to-bench-to-bedside as a model of bringing such discoveries into the clinical arena, and in doing so addresses the issues that may enhance, or complicate, such a path of discovery, as well as the impact such advances in genetics and genomics may have on the practice of clinical medicine and the role of the physician.

**Keywords:** animal models, genetics, genomics, human genome, neurogenetics, neurotransmitters, NIH Roadmap, pharmacogenomics, rare disorders, translational research

future medicine

The accompanying articles featured in this themed issue of *Future Neurology* outline and detail the progress made over the last decade in the study of the disorders of neurotransmitters. As these disorders are potentially treatable, they remain a previously unknown class of disease that are highly significant given their role in neurological deterioration in both children and adults. As is the case with the study of rare disorders, the larger challenge lies in the translation of clinical observations and basic science investigation into rational therapies that can be introduced into clinical practice and impact individual patients. The availability of advanced technologies, such as high-throughput screening (i.e., for newborn screening), gene sequencing, microarray technology (microdeletions and gene dosage) and other platforms (i.e., proteomics, metabolomics and informatics) have changed the way we investigate disease mechanisms, and in some cases, have led to the establishment of new diagnostic tests that will identify individuals with disease conditions in various stages [1]. However, the underlying mechanism of a disease may remain unknown. Genomic research may offer opportunities to study disease processes by taking advantage of the identification of clinical phenotypes, spontaneously occurring animal models of human disease (natural variations of nature) and the use of a growing armamentarium of sophisticated methods to study molecular and cellular abnormalities that accompany disease.

## National Institutes of Health Roadmap & translational research as methods to bring research from the bench-to-the-bedside

For many years, much of the effort in research involved the individual researcher investigating basic mechanisms and pathways of human disease. However, as a result of US National Institutes of Health (NIH) funding initiatives and interests of the consumers of healthcare (the patients), research is moving down a new highway. The Roadmap initiative described by Elias Zerhouni, current Director of the NIH, espouses a set of initiatives designed to accelerate the pace of medical research and its implementation into the clinical arena [2,3]. These initiatives address challenges in medicine that cannot be undertaken by a single researcher or institute, but rather foster collaborative discovery. As such, the Roadmap proposes opportunities for new pathways to discovery, including further advances and development of the disciplines of bioinformatics, imaging, molecular genetics, genomics, proteomics, metabolomics, nanotechnology, pharmacogenetics and pharmacogenomics, in addition to

the restructuring of future research teams, and re-engineering of the clinical research enterprise [2,3]. This initiative materialized on the heels of the completion of the Human Genome project [4,5] and a previous 5-year doubling of the NIH budget, completed in the financial year 2003, both of which served to fuel public expectations and accelerate the pace of scientific discovery [6]. Roadmap initiatives may enhance the development of bench-to-bedside research initiatives by encouraging the establishment of research teams comprised of scientists and physicians from varying backgrounds.

Roadmap research initiatives may enable application of advanced technology to the study of human disease and further the development of treatments based on the discovery of mechanisms or biomarkers of disease. For example, imaging biomarker analysis is the goal of the Alzheimer Disease Neuroimaging Initiative (ADNI), the largest public–private partnership whose mission is to uncover biomarkers associated with brain and memory decline that may be predictive of normal aging, mild cognitive impairment and Alzheimer's disease. This information can be coupled with genetic endophenotyping and proteomics and may enable the early identification of individuals who are at risk and who might benefit from new therapies [7,8].

Translational medicine is the term now used broadly to describe this transition from the laboratory bench of the basic scientist to the bedside or clinic of the primary-care physician [9]. This is not a new concept, and has formed the cornerstone of medical research and discovery for many decades. A great deal of medical research has occurred due to the bedside-to-bench-to-bedside model. Many medical discoveries are made at the bench of the basic scientist, who investigates the basic elements of disease using readily available scientific and laboratory tools. With the sophistication and availability of technology, and after varying periods of investigation and subsequent clinical trials, the discovery may be translated into a clinically applicable treatment, thus completing the cycle to the patient's bedside.

## Path of discovery in rare disorders

Essentially, this is how progress in rare disorders has evolved [10]. Importantly, this pathway reflects the evolution of knowledge and the elucidation of the phenotypes and biochemical pathways of the neurotransmitter disorders. A patient with a novel condition is identified in the clinic. The clinical features or phenotype are described, and other patients with similar findings are sought. Using the technologies available at that time, (body fluids, blood, urine and cerebrospinal fluid [CSF]) can be analyzed to uncover novel metabolites or biomarkers of disease [11–15]; either of two paths can unfold. The first approach may involve the molecular determination of pathophysiology using either reverse genetics or linkage analysis. An example to illustrate this is the recent discovery of *LMNA* gene mutations as the cause of Hutchinson–Gilford Progeria syndrome, a rare disorder of accelerated aging presenting in childhood and leading to premature death [16]. Since the discovery that a mutation in the *LMNA* gene causes progeria, scientists have subsequently developed several mouse models for the disorder and from these have been able to gain insight into the disease's molecular and cellular basis, such that experimental treatment strategies are now being proposed [17,18]. The challenge remains to determine whether such treatments will be both safe and effective for children with progeria. The genetic defects in the nuclear envelope protein prelamin-A or in the FACE-1 metalloprotease (also termed Zmspte24) involved in prelamin-A proteolytic maturation, lead to the accumulation of an abnormal form of this protein with eventual disruption of nuclear envelope integrity [19]. This disruption leads to alterations in chromatin organization, genomic instability, transcriptional changes and activation of a p53-linked signaling pathway. By using genetic manipulation in mouse models, lowering prelamin-A levels results in a total recovery of Zmpste24-deficient mice from the accelerated aging process.

Alternatively, study of the mechanisms of pathophysiology at the basic science level, involving either cellular or animal models, can occur and reveal pathways that might lead to gene discovery, which in turn might lead to a diagnostic test or biomarker to determine individuals at risk [15]. If a biochemical pathway is elucidated, one can establish diagnostic methods based on abnormal, excessive or deficient metabolites in the blood, urine, CSF or other tissues. High-resolution nuclear magnetic resonance spectroscopy of biofluids such as plasma, CSF or urine can generate metabolite fingerprints containing information regarding the physiological and/or pathological states of disease. The identification of biomarkers in human diseases can be used to follow disease progression and response to therapy, as well as identify presymptomatic or oligosymptomatic patients who have increased chances of response to new

therapies [20,21]. Reliable biomarkers should be quantifiable, reproducible and change with the progression of the disease or with treatment.

### Linkage analysis & gene identification

Using genome-based technologies, one can collect families with similarly affected individuals to look for similarities in the DNA structure and locate these similarities on a specific chromosome and locus. The next step involves identifying genes in that region and, based on candidate gene approaches, identify changes or mutations in the DNA that may be associated with the disease in questions, as either a marker or a true sequence variation or mutation. One can use a candidate gene approach, which is a strategy employed in order to identify disease-associated genes, based on finding candidate genes in a chromosome region in which a disorder is mapped [22]. In this way, potential disease associated mutations can be identified.

Gene identification can lead to the development of diagnostic tests, drug therapies and further research into individual variations that may alter the clinical phenotype. Such tests will enable physicians to evaluate their patients in the clinic and prescribe individualized therapies [23]. As is usually the case in clinical neurogenetics, once a molecular test becomes clinically available, one can use this to test phenotype extremes from severe or typical to more mild [24–30]. Thus, the true phenotype is no longer defined strictly by clinical criteria.

### Use of animal models to accelerate human research & prepare for preclinical trials

Animal models have greatly contributed to the current progress made in understanding disease pathogenesis [31,32] and several mouse models exist for the study of the neurotransmitter disorders [33–36]. The advantages of having a mouse model for Hutchinson–Gilford progeria syndrome has been discussed, in that the rapid advancement from positional cloning to construction of a mouse model, and to the study of the mechanisms involved, has led to the development of preclinical therapeutic agents that will be tested in human trials. The mouse has long been recognized as genetically similar to humans. In fact, the completed mouse and human genomes attest to the fact that the two genomes are at least 85% identical. Mouse models are an economic investment, as they are relatively inexpensive to maintain, and thus are used in a majority of animal model experiments. There are many sporadically occurring mutant mice that are ideal models on which to study human disease, as they are born with mutations in at least one gene. Alternatively, if the gene of interest is known, a deletion or knockout of the gene can be genetically manipulated [37]. Murine knockout models of inborn errors of metabolism are commonly employed to characterize disease pathology. When a mouse is found to be a good model for a human disease, various treatments can be evaluated and primary and secondary biomarkers as end points for treatment can be studied effectively. As a result of these preclinical investigations, therapies that appear to be promising in mouse studies may ultimately lead to the development of a therapy for humans with a similar disorder. Not every mouse model is a perfect match for its human disease counterpart. The ideal mouse model shares common clinical, biochemical and genetic features with human disease. It should also survive long enough to be able to investigate its properties and study natural history and treatments (preclinical therapies).

Despite the best intentions, some diseases do not have mouse models because the mouse is not born (embryonic lethality) or dies early in its development. In this case, a conditional knockout may be preferred in which the investigator can eliminate the gene in an organ-specific manner. In addition, one must keep in mind that various strains of mice may manifest the genetic manipulations differentially due to differences in their genetic background [37]. The development of mouse phenotyping core facilities in several universities will seek to address these complexities [38].

Other than the background effect, mice may differ from their human counterparts due to the presence of modifier genes, or other genes or regions of DNA that interact in a currently undetermined manner to alter the phenotype of the individual manifesting the gene mutation. The concept of modifiers is being given increasing attention in order to explain the genetic heterogeneity observed in humans with the same genetic condition, as well as to explain variations in response to medications and as a way to understand drug toxicities by expanding our knowledge in the field of pharmacogenomics [39–40]. From a clinical standpoint, pharmacogenomics may enable us to understand why a particular drug may be advantageous in one patient and toxic for another. This will potentially set the

stage for the implementation of individualized medical care with treatment based upon genome discoveries.

The completion of the Human Genome Project has ushered in the next era of genetic exploration – the role of variability in genetics within the human population.

## How mouse models may advance the development of treatments for human disorders

Duchenne (D) and Becker (B) muscular dystrophy (MD), X-linked neuromuscular disorders, are caused by alteration or absence of dystrophin, thus resulting in muscle membrane damage [41,42]. The pathophysiological consequences of DMD or BMD include myofiber necrosis, fibrosis and muscle wasting. Despite progress in the understanding of the pathophysiology of dystrophin deficiency, current treatments are limited. The high mutation rate of the dystrophin gene in humans also occurs in other mammals, and sporadically occurring dystrophin-deficient animal species have been identified [43]. For example, the *mdx* mouse, the first animal model of DMD to be identified, remains the best and most widely studied model organism for DMD and its treatments [43]. In addition, other animal species, including Golden Retrievers, have been found to exhibit dystrophin deficiency [44]. Several research groups studying the the *mdx* mouse model of DMD have identified potentially beneficial treatments [45]. Several of these studies have led to clinical investigations for recent human clinical trials [46].

Another example whereby the development of mouse models has been useful for translational research is the National Cancer Institute's Mouse Consortium, whose goal is the development of mouse cancer models [47]. These models, whose natural disease histories reveal novel insights about cancer initiation and progression, also respond to appropriate standard-of-care therapies in preclinical testing. Incorporation of imaging and nanotechnology approaches into preclinical testing increases the likelihood that the models will enable better stratification and randomization of patients, improved definition of surrogate end points of response, and novel approaches to noninvasive imaging.

The use of genetically modified mice to establish gene function has been well documented in the literature, and their role in drug discovery has been proved to be extremely valuable [48]. The development of the Phenotype-finder platform, by which knockout mice for specific genes of interest undergo comprehensive phenotyping using a panel of diverse assays, is a novel concept in translational research. This analysis has led to the identification of new functions for genes of interest as well as the identification of potential detrimental effect.

## Examples of progress in translating research findings into the clinical arena

Two disorders are highlighted below to summarize and illustrate the transition of advances in neurogenetic disorders to clinical practice: Rett syndrome and the neurotransmitter disorders.

### *Rett syndrome*

The identification of the *MECP2* gene in Rett syndrome has led to a change in our traditional thinking about this condition [49]. Prior to the possibility of a molecular diagnosis, the condition was defined and diagnosed on clinical grounds [50]. The clinical features were held to the original findings described by Andreas Rett, namely that of a neurological disorder affecting females displaying a clinical course defined by age- and disease-specific stages.

In the earliest stages after normal development, girls display a developmental arrest (Stage 1) followed by a regression with loss of speech and all purposeful hand use, with the concurrent appearance of postnatal microcephaly, stereotypic 'hand-washing' activities, ataxia, hand-apraxia and abnormal breathing (Stage 2). At Stage 3, there is a limited improvement with plateau, followed by a final neurological deterioration in older girls (Stage 4). However, it is now appreciated that a number of Rett syndrome variants exist and have been described [51]. Atypical milder cases are recognized [52], as are those with autism and even males with neonatal encephalopathy [51–58].

### *Neurotransmitter disorders*

Likewise, the first patients with neurotransmitter disorders were described several decades earlier with the diagnostic criteria initially defined on clinical grounds [59–67]. It has since been recognized that neurotransmitter disorders are not one disease, but rather a group of neurometabolic syndromes attributable to a primary disturbance of neurotransmitter metabolism or transport. Collectively, they can often cause severe, progressive neurological damage. Over the past decade, it has become apparent that neurotransmitter disorders

comprise an enlarging group of clinically recognized disorders requiring specialized diagnostic procedures for detection. They are potentially treatable. These disorders are characterized by defects in biopterin, catecholamines, serotonin, glycine, pyridoxine and γ-aminobutyric acid (GABA) metabolism [68]. Newly described syndromes, such as cerebral folate deficiency and pyridoxal-5-phosphate dependency, have been added to the list of conditions that may be responsible for seizures and encephalopathy in childhood [69–72].

The earliest descriptions of these conditions involved subjects with intractable seizures, movement disorders or paroxysmal neurological symptoms. Using observations obtained in the individual patients, other affected patients have been identified leading to the identification of biochemical pathways that are disrupted in addition to genetic loci. Once the molecular basis is understood, the search for patients with the same genotype can uncover the broad phenotypic spectrum. Additionally, the identification of biomarkers of disease have led to an understanding of the underlying pathways [73,74], and form the basis for diagnostic screening tests, as well as providing the basis for the development of paradigms to determine who shall go on to have focused genetic testing. The diagnosis depends upon the reliable quantification of CSF metabolites. Some experiences with specific therapy based on underlying mechanisms have been reviewed [74,75], and continues to be an ongoing area of research emphasis.

## Where do we go from here?

At the beginning of the 21st Century, we witnessed one of the largest accomplishments in science: the mapping of the human genome [4,5]. This has opened up an unchartered territory in science, one with unknown bounds. Due to this monumental task, the new challenge is to now translate the information contained in these small pieces of genetic instruction into patient care and health policy. To affect this, we must educate our entire health care network of professionals to be knowledgeable and freely conversant in the vocabularies of genetics and genomics. This information poses additional challenges and raises questions and concerns regarding informed consent and access to confidential genetic information. The possibilities for genetic discrimination, as well as potential health disparities [76], remain new issues to explore.

While the majority of genes identified as a result of the Human Genome Project are associated with rare disorders, many others have been identified that may increase susceptibility to more common diseases, such as diabetes, hypertension and heart disease. In addition, the risk for certain diseases may increase when genes interact with additional genes (modifier genes) or environmental factors, which may be physical, behavioral, chemical, infectious or nutritional in nature.

The most common effects of modifier genes are often described as being additive or multiplicative. Epistasis refers to the condition in which a genetic interaction between an allele from one gene masks the phenotype caused by a mutation or sequence alteration in another gene [77].

## New role of the clinician in the genome era

The clinically oriented physician practicing in the genome era will be expected to apply the basic concepts of genetics and genomics, including recognition of inheritance patterns, the concept of the interaction of genes with the environment, and health promotion in the care of individualized patients. Clinicians will be expected to identify resources for genetic testing and genome-based interventions.

Physicians will need to understand the risks and benefits of genomics in health and disease assessment in the context of clinical practice. For many disorders, accurate clinical diagnosis might be possible, but a patient with a mild form of a disorder (perhaps identified through an affected relative) could face uncertainty as to the diagnosis and their disease status as well as that of their at-risk offspring.

Basic and clinical research on inherited neurological disorders has already provided important information regarding disease pathogenesis, and enabled the refinement of diagnostic techniques for many disorders, several of which have been discussed. The pace of advancement in molecular genetic research is rapid, and offers the hope that treatment of genetic diseases and the genetic aspects of multifactorial disorders will one day become a part of the standard management of these conditions.

## Conclusions

An emerging set of technologies promises to revolutionize the practice of clinical medicine and facilitate the flow of information from bedside-to-bench-to-bedside and back. However,

the increasing complexity of molecular/clinical information, the barriers between clinical and basic researchers, funding issues and the lack of multi- and interdisciplinary investigators working in collaborative research teams challenge our successful translation of this knowledge. Successful translation will require a transformation of clinical research and the re-education of medical professionals.

The sequencing of the human genome, a broader understanding of the role of genes and proteins in health and disease, expansion of scientific disciplines and the emergence of technologies for analyzing molecular networks in patient samples have combined to create an exciting opportunity for discovery in medicine. These discoveries will transform the practice of medicine.

## Future perspective

The integration of genetics and genomics into clinical practice, public health research and policy will continue to be one of the most important challenges in healthcare. The next decade and beyond will provide the opportunity to establish the critical infrastructures that will enable scientific advances to be translated into medical interventions that may impact health and disease, by allowing the formation of collaborative research networks that span multiple disciplines (including biological, computation, imaging and drug development), which will ultimately spur the development of specific therapies, which will not only address the single gene disorders, but also take into account human variability and response to environmental regulation and response to medications.

## Executive summary

***Progress of neurogenetic disorder investigation***

- A novel disorder is described in the clinic.
- Search undertaken for patients who share similar clinical features.
- Body fluids and accessible tissues are used to locate disease-related biomarkers.
- This may lead to the understanding of pathophysiology or biochemical pathways underlying the disorder.
- Development of a diagnostic test is likely to follow.

***Alternative molecular genetics approach and application of linkage analysis & candidate gene identification***

- In this way, the mutation can be identified and used to develop a diagnostic test, or one can search for expansion of the phenotype and recognition of a milder disease spectrum.
- Once the gene is known, scientific investigation can progress through the development and use of animal models, basic research on tissues, imaging and molecular genetics to further probe pathophysiology and/or test therapies that might be brought to the clinical bedside in future.

***Preclinical development: two important accomplishments***

- The US National Institutes of Health Roadmap initiative, which promotes collaborative research teams and integration of genetics, genomics, pharmacogenomics, imaging, computational strategies and so on.
- The sequencing of the human genome and the focus on identifying regions of the genome that play a regulatory role.

***Disease identification & delineation of clinical phenotypes***

- With the discovery of DNA alterations, one can assess the ends of the phenotype, including milder individuals.
- This has led to a reinterpretation of the clinical definition of Rett syndrome and continues to help identify patients with the rare and new class of disorders collectively referred to as the neurotransmitter disorders.

***The genome era will demand an important skill set***

- The clinical physician practicing in the genome era will be expected to apply the basic concepts of genetics and genomics, including inheritance patterns, the interaction of genes and the environment and health promotion in the care of individualized patients.
- Clinical physicians will be expected to identify resources for genetic testing and genome-based interventions.
- Physicians will need to understand the risks and benefits of genomics in health and disease assessment in the context of clinical practice.

***Future perspective***

- The integration of genetics and genomics into clinical practice, public health research and policy will continue to be one of the most important challenges in healthcare.

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