Neurogenetics

Five new things

Suman Jayadev, MD

Corrine O. Smith, MS

Thomas D. Bird, MD

Summary

Clinical neurology has benefitted greatly from recent remarkable advances in molecular genetics. In 1991, we could approximate a patient's risk for Huntington disease (HD) based only on linkage analysis. Now, 20 years later, not only can we identify the HD mutation with certainty, we can do the same with several hundred diseases. Whole genome or exome sequencing will soon allow for one-step interrogation of multiple genes for an even larger range of diseases. The recognition of these genes and their associated proteins in combination with new technology has led to creative new approaches to treatment. The challenge for the practicing neurologist is to provide clinically relevant and accurate interpretation of the genetic test results, with successfully treating once "incurable" neurogenetic diseases our ultimate goal.

The tremendous advances in the field of human genetics over the last 3 decades have been referred to as a genetic revolution. In actuality it has been a progressively developing evolution in which knowledge of molecular biology has advanced hand in hand with new technology. Two major landmarks were the first identification of human disease-associated genes in the mid-1980s and the sequencing of the human genome in 2001. These advances have directly affected the practice of medicine in general and the practice of neurology in particular. Here we briefly discuss 5 new things in the field of neurogenetics.

New genes, new diseases, new mechanisms

The first neurologic disease to have its underlying gene identified was Duchenne muscular dystrophy in

Departments of Neurology (SJ, COS, TDB) and Medicine (COS, TDB), University of Washington, Seattle; and Geriatric Research Education and Clinical Center (TDB), VA Puget Sound Health Care System, Seattle, Washington.

Correspondence to: tomnroz@uw.edu

DNA segments can now be sequenced in parallel in high throughput formats, allowing for the read of millions of base pairs in hours.

1986. Many more followed. In some cases, disease-causing mutations have been found in genes associated with well-established disease entities such as Huntington disease, myotonic muscular dystrophy, Charcot-Marie-Tooth (CMT) neuropathy, and Friedreich ataxia. These rare Mendelian diseases with high penetrance are shown on the left side of figure 1. Trinucleotide repeat expansions and gene duplication and deletion are other ways in which genes can be mutated. In other cases, entirely new diseases have been described on the basis of combining relatively unique phenotypes with their associated genetic basis. Examples of these new diseases are 1) PHARC (peripheral neuropathy, hearing loss, ataxia, retinitis pigmentosa, cataract) associated with mutations in the *ABHD7* gene¹; 2) sensory neuropathy, dementia, and hearing loss caused by mutations in the DNA methyltransferase 1 gene²; and 3) frontotemporal dementia/amyotrophic lateral sclerosis caused by an expanded nonexpressed hexanucleotide repeat in the *C9orf72* gene.^{3,4} New genetic mechanisms underlying diseases have been discovered such as the derepression of the *DUX4* gene in FSH muscular dystrophy⁵ and a group of diseases, such as myotonic dystrophy, caused by abnormalities in RNA processing.⁶ We now know that a single phenotype such as cerebellar ataxia or epilepsy can be caused by mutations in more than 30 different genes and, conversely, that different mutations in a single gene can cause numerous phenotypes. One example is the *LMNA* gene encoding lamin A/C, where both autosomal dominant and recessive mutations exist and can manifest in a diverse array of phenotypes including Emery-Dreifus muscular dystrophy, congenital muscular dystrophy, CMT, Hutchinson-Gilford progeria, or dilated cardiomyopathy.

In many instances the newly identified genes code for proteins whose normal functions remain unclear, e.g., amyloid precursor protein associated with Alzheimer disease (AD), prion protein associated with familial Creutzfeldt-Jacob disease, and huntingtin protein associated with HD. Identifying the normal functions of these proteins and the ways in which mutations disturb these functions represent major efforts in biomedical science. However, the normal

Figure 1 Spectrum of the effect of genetic variation

Genetic diseases and their associated genes can be divided into penetrance (from high to low on the y-axis) and frequency (very rare to common on the x-axis). Single gene Mendelian diseases (such as Huntington disease and muscular dystrophies) are rare, but have high penetrance. Diseases such as Alzheimer disease, Parkinson disease, and stroke are common, but their related risk genes have low penetrance. (From McCarthy MI, Abecasis GR, Cardon LR, et al. Genome-wide association studies for complex traits: consensus, uncertainty, and challenges. *Nat Rev Genet* 2008;9:356-369. Reprinted with permission from Macmillan Publishers Ltd: Nature Reviews Genetics.)

Figure 2 GeneTests: Growth of laboratory directory

From 1993 to 2010 there has been a considerable increase in the number of genetic testing laboratories and the volume of genetic diseases for which testing is available. (From www.genetests.org, 2011.)

function of the wild-type protein may be obscure or perhaps irrelevant, as it may be the toxicity of the mutated version of the gene that causes disease. It is this further understanding that will pave the way toward treatments.

Clinical genetic testing

Discovery of new disease-associated genes has led to the clinical application of genetic testing. The explosion in genetic testing is shown in figure 2. In 1995, there were approximately 10 commercially available genetic tests relevant to neurology. Now there are several hundred tests related to all areas of clinical neurology including neuromuscular disorders, dementias, movement disorders, stroke, and white matter diseases. The availability of genetic testing has led to the expansion of disease phenotypes. For example, “senile chorea” has now been found generally to represent late-onset Huntington disease and a late-onset tremor ataxia phenotype (*FXTAS*) is now known to be part of the spectrum of the fragile X mental retardation syndrome. In addition, chromosomal microarray studies can now identify deletions or duplications in the genome (also known as copy number variants) often associated with childhood developmental disabilities or congenital brain anomalies. These studies have helped identify *SHANK2* and *SHANK3*, synaptic scaffolding genes, as playing a role in autism.⁷ Microarray studies are now considered first-tier tests for individuals with developmental disabilities.⁸

The use of these genetic tests is complicated. The selection of appropriate tests depends on a detailed knowledge of differential diagnosis, relative frequency of disease subtypes in the population, test costs, test availability, and time required for the analysis. Attention must also be paid to the reasons for ordering the test. Sometimes a genetic test is used to make a specific diagnosis in an affected person. In fact genetic test results may represent the gold standard for a clinical diagnosis. Alternatively, the test may be used to identify a disease-associated mutation occurring in an asymptomatic family member who is at risk for the disease. Finally, interpretation of genetic test results can be problematic. Testing often reveals DNA changes whose clinical significance is unclear (variants of unknown significance). For these reasons genetic testing frequently requires the expertise of specialists in genetic medicine and genetic counseling. In some cases, consultation with genetic medicine specialists or genetic counselors

In 1995, there were approximately 10 commercially available genetic tests relevant to neurology. Now there are several hundred tests related to all areas of clinical neurology.

may not be feasible or timely. When general neurologists pursue genetic testing, we recommend testing the most likely gene candidates first (which can often be identified through reviews in www.genetests.org). Some laboratories do have reflexive panels through which testing can be tiered; most common genes are tested first followed by more exhaustive gene testing if initial tests are negative. Genetic testing can raise complex issues regarding insurance, employment, and other legal, emotional, and socioeconomic factors. The US federal government has recently taken a significant step in protecting individuals from discrimination based upon genetic testing and information. The Genetic Information and Nondiscrimination Act of 2008 (GINA) took effect in 2009. GINA states that individuals may not be discriminated against by employers or health insurers based upon genetic information. There are important exclusions to this rule: 1) life, disability insurance, and long-term care are not included; 2) individuals in the US military, the Veterans Administration, and Indian Health Service systems are not protected; and 3) symptomatic individuals protected by the Americans with Disabilities Act are excluded. Clearly the merging of new genetic testing technology into our daily life logistics is challenging. Other countries are pursuing their own approaches to these issues, though discussion of alternatives is beyond the scope of this review.

Whole genome and whole exome sequencing

Sequencing of the first entire human genome required nearly a decade and 3 billion dollars. Recent technical advances have been so remarkable that the cost is now a few thousand dollars and the technology will soon be available in the practice of medicine. Next-generation sequencing technologies have overtaken traditional Sanger sequencing methods in the study of genome variation. DNA segments can now be sequenced in parallel in high throughput formats, allowing for the read of millions of base pairs in hours—about 100 times faster than earlier approaches used less than 10 years ago. Proof-of-principle studies have demonstrated that whole genome sequencing (WGS) can be successfully employed to identify causative mutations in previously known genes associated with neurologic diseases such as CMT.⁹ Alternatively, sequencing can be limited to the coding regions of the human genome (exome sequencing) which represents 2% of the entire genome. This approach has led to the identification of a new gene (*DYNC1H1*) associated with a dominant form of CMT2.¹⁰ Since the majority of monogenic diseases caused by single base variations are predicted to occur in exons, whole exome sequencing may be a more feasible approach. Exome sequencing is also being used in research to identify rare or new mutations associated with genetically complex diseases such as autism and AD as well as rare single gene Mendelian diseases. The clinical use of this technology will likely have important consequences for practicing neurologists. Genetic testing may become more straightforward and less expensive. Rather than having to select from a menu of hundreds of genes the clinician may be able to order one test that covers many disease subtypes and all the relevant genes. However, interpretation of the results may be extremely difficult. More variants of unknown significance will be identified; consequently it will be difficult to separate the disease-causing mutation from the genetic background noise

(normal variants and polymorphisms). This has been referred to as “the thousand dollar genome with the million dollar analysis.” This interpretation problem will require a great deal of work and attention in molecular analysis.

Genome-wide association studies

Most of the newly discovered genes and diseases discussed here are single gene disorders showing classic Mendelian inheritance patterns of autosomal dominant, autosomal recessive, or X-linked. They are usually rare but have high penetrance. While these causative genes have been successfully identified, investigators are now seeking to recognize the gene variants that may confer risk to disease, perhaps in conjunction with other genetic variants or environmental influences. This has been conceptualized as a spectrum of the effect of genetic variation (figure 1). The monogenic diseases following Mendelian inheritance patterns tend to be caused by highly penetrant and rare mutations, whereas more common variants or polymorphisms (presenting with at least 1%–5% frequency in the general population) may contribute to complex genetic diseases such as sporadic AD and autism spectrum diseases.

Genome-wide association studies analyze subsets of the 3 million or so DNA polymorphisms that make us each individuals. By testing 1 million or so single nucleotide polymorphisms (SNPs) in a single test of an individual's DNA, the pattern seen in patients with a common disease (e.g., AD, multiple sclerosis, macular degeneration) can be compared to normal controls, and patterns of polymorphisms associated with the disease state. The polymorphisms associated with the disease are often not pathogenic, but just mark a region of the chromosome where a certain variant of that region likely predisposes to disease. However, in some cases the SNPs can be directly pathogenic. In contrast to whole genome or exome sequencing, genotyping is not performed nucleotide by nucleotide, but only at the SNP locations.

The results of these GWAS studies have been successful. That is, genetic variants have been identified and confirmed that are clearly associated with specific diseases. For example, 10 such genes in addition to *APOE* have been found to be associated with AD.¹¹ However, the apparent contribution of these genes to the pathophysiology of the associated diseases is relatively small. The incurred risk factors are generally not large enough to be useful with our current technology in the clinic setting. Nevertheless, the identification of these genes and their associated proteins should lead to a better understanding of the biological causes of the diseases.

New neurogenetic therapeutics

It is clear that the leap from disease gene discovery to therapy is daunting. For decades we have treated some neurogenetic diseases without a molecular approach such as the dietary treatments of PKU and Refsum disease and the replacement of pyridoxine in children with pyridoxine-dependent seizures. Now replacement of the abnormal protein with normal protein can be highly beneficial as demonstrated by protein replacement therapy in Pompe (acid maltase deficiency) muscular dystrophy.¹² Development of new technologies and delivery methods has broadened our therapeutic approaches to genetic diseases. There are now a multitude of intriguing ideas on how to potentially treat neurogenetic diseases that may prove to be of great benefit in the near future. One such option is gene replacement therapy, which is being pursued in both animal models and humans with

Neurogenetics: Five new things

- The discovery of new genes, diseases, and mechanisms has led to a greater understanding of disease pathogenesis.
- Availability of genetic testing has led to expansion of disease phenotypes.
- Whole genome and exome sequencing will likely make genetic testing less expensive but add layers of complexity to test interpretation.
- Genome-wide association studies have led to the discovery of genetic variations clearly associated with specific diseases.
- Gene replacement, stem cells, and RNA silencing are new approaches in neurogenetic therapeutics.

Figure 3 A potential therapeutic approach to myotonic muscular dystrophy (DM1)

Myotonic dystrophy type 1 can result from muscle blind protein (MBNL1) binding to the hairpin RNA produced by the abnormal CTG expansion present in DM1 thereby preventing the normal splicing of other genes (such as that coding for the chloride channel [CLCN1] resulting in myotonia). Normal function could be restored by using an antisense oligonucleotide (CAG25) to displace the muscle blind/RNA binding and free the RNA to be exported to the cytoplasm. (From Cooper TA. Molecular biology: neutralizing toxic RNA. *Science* 2009;325:272. Reprinted with permission from AAAS.)

Duchenne muscular dystrophy.¹³ Another possibility is antisense oligonucleotide therapy that is currently in clinical trials for Duchenne muscular dystrophy. Figure 3 shows how this might be accomplished as demonstrated in a mouse model of myotonic dystrophy (DM1).¹⁴ Yet another approach is called RNA silencing or interference in which RNA produced by the mutated gene coding the abnormal protein is silenced or destroyed allowing the normal gene and protein (present in persons with autosomal dominant disorders) to pursue its normal function.¹⁵

Increased understanding of how mutant proteins produce malfunction of the relevant biochemical pathways has led to new ideas for treating 2 common neurogenetic diseases of childhood. The first is tuberous sclerosis (TSC) in which mutations in 2 tumor suppressor genes (hamartin and tuberin) fail to suppress mTOR leading to abnormal cellular growth. Successful treatment of subependymal giant cell astrocytomas in TSC patients with Everolimus, which inhibits mTOR, has been reported.¹⁶ The second is fragile X syndrome (FXMR), in which the abnormal fragile X-related protein (FMRP) fails to inhibit overexpression of metabotropic glutamate receptors. Clinical trials are now studying whether mGluR5 antagonists will benefit patients with FXMR.¹⁷

The field of stem cell therapeutics is clearly exciting but cannot be reviewed here in detail. An example of the potential of this approach is the successful treatment of the autosomal recessive leukodystrophy in Shiverer mice with pluripotent stem cells with implications for human pediatric myelin disorders.¹⁸

Practicing neurologists should follow these unfolding events with fascination and enthusiasm. Through continuing education we need to stay up-to-date on the latest developments in neurogenetics in order to provide our patients with the best diagnosis, care, and management.

APPENDIX

Chromosomal microarray testing

A specialized test of patient DNA used for the detection of duplicated or deleted region of DNA that could cause a phenotype or disease.

Copy number variation

Any duplicated or deleted segment of DNA. Copy number variations may be benign or pathogenic depending on size and location in the genome.

Exome

Collective regions of DNA (exons) that are “expressed” into proteins.

Genome-wide association study

The unbiased, discovery approach to determining whether gene polymorphisms are associated with risk of disease.

Next generation sequencing

Technologies focused on sequencing DNA using high throughput programs that include “massively parallel sequencing” or multiple sequencing reactions. New technologies now cut DNA into many small pieces then sequence them many times.

Single nucleotide polymorphism

A polymorphism, or gene variant that involves only 1 nucleotide such as a change from C to T in the DNA sequence. Polymorphisms occur with high prevalence in the general population (at least 1%–5%). There are over 1 million SNPs thus far identified in the human genome.

Whole genome sequencing

A process that sequences base by base the entire genome of an individual. This method includes sequencing not only exomes, but also introns and nongene-associated DNA regions.

REFERENCES

1. Fiskerstrand T, Brahim DHB, Johansson S, et al. Mutations in *ABHD12* cause the neurodegenerative disease PHARC: an inborn error of endocannabinoid metabolism. *Am J Hum Genet* 2010;87:410–417.
2. Klein CJ, Botuyan MV, Wu Y, et al. Mutations in *DNMT1* cause hereditary sensory neuropathy with dementia and hearing loss. *Nat Genet* 2011;43:595–600.
3. DeJesus-Hernandez M, Mackenzie IR, Boeve BF, et al. Expanded GGGGCC hexanucleotide repeat in noncoding region of C9orf72 causes chromosome 9p-linked FTD and ALS. *Neuron* Epub 2011.
4. Renton AE, Majounie E, Waite A, et al. A hexanucleotide repeat expansion in C9orf72 is the cause of chromosome 9p21-linked ALS-FTD. *Neuron* Epub 2011.
5. Van der Maarel SM, Tawill R, Tapscott SJ. Facioscapulohumeral muscular dystrophy and DUX4: breaking the silence. *Trends Mol Med* 2011;17:252–258.
6. Todd PK, Paulson HL. RNA-mediated neurodegeneration in repeat expansion disorders. *Ann Neurol* 2010;67:291–300.
7. Berkel S, Marshall CR, Weiss B, et al. Mutations in the SHANK2 synaptic scaffolding gene in autism spectrum disorder and mental retardation. *Nature Genet* 2010;42:489–491.
8. Miller DT, Adam MP, Aradhya S, et al. Consensus statement: chromosomal microarray is a first-tier clinical diagnostic test for individuals with developmental disabilities or congenital anomalies. *Am J Hum Genet* 2010;86:749–764.
9. Lupski JR, Reid JG, Gonzaga-Juregui C, et al. Whole-genome sequencing in a patient with Charcot-Marie-Tooth neuropathy. *N Engl J Med* 2010;362:1181–1191.
10. Weedon MA, Hastings R, Caswell R, et al. Exome sequencing identifies a DYNC1H1 mutation in a large pedigree with dominant axonal Charcot-Marie-Tooth disease. *Am J Hum Genet* 2011;89:308–312.
11. Naj AC, Jun G, Beecham GW, et al. Common variants at MS4A4/MS4A6E, CD2AP, CD33 and EPHA1 are associated with late-onset Alzheimer's disease. *Nat Genet* 2011;43:436–441.
12. Kishnani PS, Corzo D, Nicolino M, et al. Recombinant human acid α -glucosidase: major clinical benefits in infantile-onset Pompe disease. *Neurology* 2007;68:99–109.
13. Goyenvalle A, Seto JT, Davies KE, Chamberlain J. Therapeutic approaches to muscular dystrophy. *Hum Mol Genet* 2011;20:R69–R78.

14. Wheeler TM, Sobczak K, Lueck JD, et al. Reversal of RNA dominance by displacement of protein sequestered on triple repeat RNA. *Science* 2009;325:336–339.
15. Gonzalez-Alegre P, Paulson HL. Technology insight: therapeutic RNA interference: how far from the neurology clinic? *Nat Clin Practice Neurol* 2007;3:394–404.
16. Krueger DA, Care MM, Holland K, et al. Everolimus for subependymal giant-cell astrocytomas in tuberous sclerosis. *N Engl J Med* 2010;363:1801–1811.
17. Hampson DR, Adusei DC, Pacey LKK. The neurochemical basis for the treatment of autism spectrum disorders and fragile X syndrome. *Biochem Pharmacol* 2011;81:1078–1086.
18. Goldman SA. Progenitor cell-based treatment of the pediatric myelin disorders. *Arch Neurol*. 2011;68:848–856.

DISCLOSURES

Dr. Jayadev serves as a consultant for About.com. C.O. Smith reports no disclosures. Dr. Bird serves on scientific advisory boards for the Association for Frontotemporal Dementia and the CMT Association; has received funding for travel and speaker honoraria from Athena Diagnostics, Inc.; serves on the editorial boards of *Brain* and *Neurology Today*; is listed as a co-inventor on and receives license fees from Athena Diagnostics, Inc. for patents re: PKCgamma in diagnosis of SCA14 and Mutations Associated With A Human Demyelinating Neuropathy (Charcot-Marie-Tooth Disease Type 1C); receives royalties from the publication of *Human Genetics: Principles and Approaches*, 4th ed. (Springer-Verlag GmbH Biomedical Sciences, 2010); serves on the speakers' bureau for Athena Diagnostics, Inc.; and receives research support from the Department of Veterans Affairs.

Related articles from other AAN physician and patient resources

Neurology® • www.neurology.org

An algorithm for genetic testing of frontotemporal lobar degeneration.

February 1, 2011;76:475–483.

Editorial: Huntington disease: A tale of two genes.

September 23, 2009;73:1254–1255.

A neurologist's guide to genome-wide association studies.

February 10, 2009;72:558–565.

Genetics of familial amyotrophic lateral sclerosis.

January 8, 2008;70:144–152.

Parkinson disease, 10 years after its genetic revolution: Multiple clues to a complex disorder.

November 27, 2007;69:2093–2104.

Continuum® • www.aan.com/go/elibrary/continuum

Neurogenetics.

April 2011

Neurology Now® • www.neurologynow.org

The hunt for genes and cures.

March/April 2007;3:20–27.

Neurology Today® • www.neurotodayonline.com

Mutants identified that implicate new pathway for multiple forms of ALS.

September 1, 2011;11:50–51.

When to test for AD genetics: New guidelines offer guidance.

August 18, 2011;11:1,30–31.

Neurology® Clinical Practice

Neurogenetics: Five new things

Suman Jayadev, Corrine O. Smith and Thomas D. Bird

Neurol Clin Pract 2011;1;41-48

DOI 10.1212/CPJ.0b013e31823c0f5f

This information is current as of December 1, 2011

Updated Information & Services	including high resolution figures, can be found at: http://cp.neurology.org/content/1/1/41.full.html
References	This article cites 16 articles, 2 of which you can access for free at: http://cp.neurology.org/content/1/1/41.full.html#ref-list-1
Citations	This article has been cited by 1 HighWire-hosted articles: http://cp.neurology.org/content/1/1/41.full.html#otherarticles
Subspecialty Collections	This article, along with others on similar topics, appears in the following collection(s): All Clinical Neurology http://cp.neurology.org/cgi/collection/all_clinical_neurology All Education http://cp.neurology.org/cgi/collection/all_education All Genetics http://cp.neurology.org/cgi/collection/all_genetics All Practice Management http://cp.neurology.org/cgi/collection/all_practice_management Models of care http://cp.neurology.org/cgi/collection/models_of_care
Errata	An erratum has been published regarding this article. Please see next page or: /content/2/1/4.1.full.pdf
Permissions & Licensing	Information about reproducing this article in parts (figures, tables) or in its entirety can be found online at: http://cp.neurology.org/misc/about.xhtml#permissions
Reprints	Information about ordering reprints can be found online: http://cp.neurology.org/misc/addir.xhtml#reprintsus

Neurol Clin Pract is an official journal of the American Academy of Neurology. Published continuously since 2011, it is now a bimonthly with 6 issues per year. Copyright Copyright © 2011 by AAN Enterprises, Inc.. All rights reserved. Print ISSN: 2163-0402. Online ISSN: 2163-0933.

involvement likely including the thalami bilaterally to also account for her coma. Furthermore, if these lesions were indeed present, they would likely be picked up on CT scan, given that her symptomatology was progressive for 3 days prior to obtaining the study. Based on our experience, the clinical recovery began too rapidly for metabolic encephalopathies causing changes in the basal ganglia.

Disclosures: See original article for full disclosure list.

Correspondence to: Hocker.Sara@mayo.edu

1. Hocker S, Rabinstein AA. Cefepime neurotoxicity can mimic postanoxic coma with myoclonic status epilepticus. *Neurol Clin Pract* 2011;1:73–74.
2. Fishbain JT, Monahan TP, Canonico MM. Cerebral manifestations of cefepime toxicity in a dialysis patient. *Neurology* 2000;55:1756–1757.
3. Dixit S, Kurle P, Buyan-Dent L, Sheth RD. Status epilepticus associated with cefepime. *Neurology* 2000;54:2153–2155.

CORRECTION

Neurogenetics: Five new things

In the article “Neurogenetics: Five new things” by S. Jayadev et al. (*Neurology® Clinical Practice* 2011;1:41–48), there is an omission in figure 2, which should read “The copyright for this figure is held by the University of Washington.” The editorial staff regrets the omission.

CORRECTION

Treating patients with medically resistant epilepsy

In the article “Treating patients with medically resistant epilepsy” by Gregory L. Krauss and Michael R. Sperling (*Neurology® Clinical Practice* 2011;1:14–23), there is an error in the second to last line in table 3. “Corpus callosotomy (anterior 66% to 80%)” should be listed as another bullet point under treatment for “Severe symptomatic forms of epilepsy” below “Third-line adjunctive therapies.” The publisher regrets the error.