Targeted DNA Sequencing for Neurological Conditions

Genetic involvement in the pathogenesis of neurological disease is significant. Among the approximately 6,400 phenotypic entries in cataloged inherited human diseases, central nervous system disorders account for approximately 60 percent, and peripheral nervous system disorders for approximately 15 percent.

For the past decade, next-generation sequencing (NGS) has been used to identify genetic causes for neurological conditions. In patients with nonspecific neurological disorders — such as children with developmental delay, where an extensive number of genetic differentials exist — whole-genome sequencing (WGS) and whole-exome sequencing (WES) have shown promise in identifying genetic causes. For patients diagnosed with a focused disease category such as inherited neuropathy, myopathy, neuromuscular junction disease, or motor neuron or epilepsy syndromes, targeted NGS panels can be efficacious and cost-effective. However, choosing appropriate genes for targeted NGS panels requires strong clinical expertise as well as genetic knowledge.

To facilitate diagnosis of genetic neurological diseases, Mayo Clinic in Rochester, Minnesota, has launched a neurology genomics clinic. In collaboration with Mayo Clinic’s Center for Individualized Medicine, the neurology genomics clinic brings together clinicians, laboratory geneticists, bioinformaticians and genetic counselors to enhance treatment and research of genetic neurological conditions.

“At Mayo Clinic, we talk about matching the right patient with the right doctor. Similarly, we want to match the right patient with the right test,” says Christopher J. Klein, M.D., a consultant in Neurology at Mayo Clinic in Rochester, Minnesota. “When patients come to us with genetic conditions, including complex neurological

Figure 1. A. MRIs demonstrate moderate to severe diffuse parenchymal volume loss, somewhat disproportionately affecting the brainstem and cerebellum. There is considerable apparent white matter loss of the cerebral hemispheres, and faint ill-defined periventricular white matter signal abnormality. B. Two heterozygous variants were identified in the FA2H gene, consistent with a diagnosis of autosomal recessive spastic paraplegia type 35.
conditions, we establish a detailed phenotype,” says Ralitza H. Gavrilova, M.D., a consultant in Neurology and Clinical Genomics at Mayo Clinic’s campus in Minnesota. “After genetic testing we deliver a diagnosis, recommend treatment and offer screening and genetic counseling for family members as needed.”

Collaboration between clinic and lab

Establishing a specific genetic diagnosis (Figure 1) can avoid the use of improper therapies as well as additional costly and invasive testing such as biopsy. In the neurology genomics clinic, patients have complete neurological examinations, including electromyography, electroencephalography, MRI, nerve-conduction tests and tests of muscle, nerve and brain pathology, as appropriate. Neurologists and laboratory geneticists discuss the results and arrive at a recommendation for testing each individual patient (Figure 2).

“The phenotypic presentations for patients with neurogenetic disorders are diverse and often overlapping. We work closely with our clinical colleagues to make sure that, based on the patient’s phenotype, the right type of testing has been ordered,” says Kevin C. Halling, M.D., Ph.D., a consultant in Laboratory Genetics at Mayo Clinic’s campus in Minnesota. “We also work closely with our clinicians in the interpretation of the NGS results.”

WGS analyzes an entire genome, composed of approximately 3 billion base pairs of DNA sequence. WES covers all protein-coding regions of the human genome, totaling about 50 million base pairs or 1.5 percent of the genome. Both procedures can produce a large number of genetic variants of unknown significance and, despite the nomenclature, have incomplete coverage of genes and exons. Targeted NGS panels start with the capture of a set of disease-focused genes followed by massive parallel sequencing. A targeted panel might only cover up to several million base pairs, and approximately 50 to 300 genes.

Nevertheless, a well-targeted panel can yield a diagnostic rate similar to WES. Mayo Clinic is developing NGS panels for peripheral neuropathy, epilepsy and neuromuscular disorders. The inherited neuropathy algorithm uses multiple criteria, including age of symptom onset, specific sensory features, ataxic or pyramidal features and nerve conduction studies.

“If a certain phenotype has a couple of hundred genes with high potential for causing disease, it makes sense to focus on them with a targeted panel,” Dr. Klein says.

When multiple genetic factors appear to be involved, WES might be recommended. If NGS finds rare functional variants in novel genes, the case is further investigated.

“When we suspect a new genetic mutation, we can collect samples from family members. Then, using WGS and functional studies, we can try to confirm that those genes are causing the problem,” Dr. Gavrilova says. “We can make a diagnosis, recommend screening for family members, including prenatal screening, and work with genetic counselors to educate patients.”

For patients, the neurology genomics clinic provides testing, diagnosis, treatment, counseling and research beneath a single roof. “Patients are happy most of all to have a diagnosis,” Dr. Gavrilova says. “They also are generally excited to participate in research. We form a very strong bond with the families we treat.

“With the resources of the neurology genomics clinic, we are connecting with researchers and different centers,” she adds. “We are creating a network that ultimately will benefit patients with these diseases.”

Figure 2. Whole-exome next-generation sequencing (NGS) is best for “diagnostic odyssey” cases where neurological localization is not well-established or genetic differential is not limited to any specific disease category. Disease-targeted NGS provides the best performance metrics for conditions with a defined genetic differential. Single-gene testing remains effective for neurological phenotypes with very specific characteristics. Image reprinted with permission from Mayo Clinic Proceedings.
Deep brain stimulation (DBS) of the subthalamic nucleus (STN) has been shown to be an effective treatment for movement disorders, including Parkinson’s disease, essential tremor and dystonia. More recently, DBS has emerged as a treatment option for Tourette syndrome. However, the exact mechanisms that lead to successful DBS treatment remain undefined.

At Mayo Clinic in Rochester, Minnesota, neurologists and neurosurgeons offer DBS for selected patients while also researching the procedure’s therapeutic mechanism, with a goal of personalizing and improving treatment. Mayo Clinic’s large patient volumes and multidisciplinary approach provide a breadth of experience to enhance patient treatment and research efforts.

“DBS is not a cure for these movement disorders, and while the results of treatment aren’t perfect, many patients have excellent results and for some, DBS can be life-changing,” says Bryan T. Klassen, M.D., a consultant in Neurology at Mayo Clinic in Rochester, Minnesota.

“We have come so far,” adds Kendall H. Lee, M.D., Ph.D., a consultant in Neurologic Surgery at Mayo Clinic’s Minnesota campus. “Our research findings are helping us to better understand how DBS works and how to refine stimulation in our patients to ease symptoms of movement disorders.”

**Multidisciplinary patient selection**

DBS is considered an option for patients with movement disorders refractory to medication. However, patient selection is key. Those with Parkinson’s disease should be experiencing shorter duration of therapeutic effect from medication or medication intolerance after initial improvement of symptoms. Cognitive impairment is generally a contraindication for the surgery; speech and balance problems usually don’t respond to DBS.

At Mayo Clinic, after initial evaluation by a neurologist, patients considered possible candidates for DBS have MRI, speech pathology testing, cognitive assessment and evaluation by a neuropsychiatrist, and consultation with a neurosurgeon. “We have a very integrated approach, and we’re all in the same building,” Dr. Klassen says.

A multidisciplinary committee discusses the test results to determine whether DBS is appropriate. “With our long experience, we understand the profile of a good candidate for DBS, and what we can expect from treatment of that particular patient,” Dr. Klassen says.

The multidisciplinary approach continues in the operating room, where the patient’s neurologist is present alongside the surgeon. “As neurologists, we have context for the patients — knowing how they functioned in the clinic and seeing how they respond to various voltage settings during surgery,” Dr. Klassen says.

Although DBS doesn’t always provide complete relief from symptoms, the outcomes can be striking. Dr. Lee cites the use of DBS for essential tremor: Micro electrode recording in the brain area can isolate what Dr. Lee calls “the tremor cells,” facilitating correct application of DBS and, commonly, the cessation of tremor in the operating room. “We discover what is abnormal in the brain activity,” Dr. Lee says, “and we stop it.”

**Searching for biomarkers**

Mayo Clinic is pursuing several lines of research to understand the therapeutic mechanisms of DBS. One effort involves determining whether DBS evokes neurotransmitter changes — specifically, dopamine release. For the past decade, the Mayo Clinic Neural Engineering Laboratory has worked to develop a device that measures neurochemical changes in response to DBS. The ultimate goal is to drive changes in stimulation parameters based on stimulation-evoked neurochemical changes.

Funded in part by a grant from the National Institutes of Health Brain Research through Advancing Innovative Neurotechnologies (BRAIN) Initiative, the researchers have tested a wireless system (Figure) in laboratory animals as well as patients undergoing DBS surgery. In a study published in the June 1, 2016, edition of *The Journal of Neuroscience*, the researchers documented a peak in dopamine release in the caudate and putamen when stimulating the dorsolateral posterior border of the STN in nonhuman primates. The findings suggest that STN DBS provokes dopamine release and that its concentration varies depending on the site being stimulated.

“*This novel system allows us to sense neurotransmitters in real time, and see exactly where in the brain we’re activating dopamine,*” Dr. Lee says. “It’s leading toward a closed-loop system.”

A second line of research seeks an open-loop system. Daniel Kruse, M.D., a consultant in Neurologic Surgery at Mayo Clinic in Rochester, Minnesota, along with Dr. Klassen, has worked to develop a device that measures dopamine concentration in vivo and displays the data graphically in nearly real time.

**Figure.** Mayo Clinic’s Wireless Instantaneous Neurotransmitter Concentration Sensing System (WINCS) measures extracellular neurotransmitter concentration in vivo and displays the data graphically in nearly real time.
electrophysiological biomarker to guide changes in stimulation parameters for DBS implants. Dr. Klassen is participating in clinical trials of a Medtronic device that delivers DBS therapy while simultaneously sensing and recording brain wave activity in Parkinson’s disease patients.

“Once we understand more about why DBS works, we can enhance the effectiveness of the settings and lower the amount of stimulation given to patients,” Dr. Klassen says. “Lower stimulation settings may mean fewer side effects, because they result from electrical current spreading to structures other than the ones we want to stimulate.

“We’ve come to the point where we’re very accurate at placing these electrodes,” he adds. “When we know more about why DBS works, we can make the systems even smarter.”

For more information

Strategy to Prevent Chemotherapy-Induced Neuropathy

Platinum drugs such as cisplatin are among the most widely used chemotherapy medications. However, approximately 40 percent of cancer patients treated with platinum drugs develop chemotherapy-induced peripheral neuropathy — generally involving loss of feeling or intense, burning or tingling pain in the feet. To date, this side effect is unpreventable and can limit chemotherapy dosage for cancer patients. Protective strategies are complicated by the possibility that preventing peripheral neuropathy might inhibit the cancer-fighting properties of the chemotherapy drug. That problem might be avoided if a protective agent were neuron targeted and delivered directly into the nervous system.

Researchers at Mayo Clinic in Rochester, Minnesota, have shown the potential of mesenchymal stem cells to act as therapy for nervous system diseases by carrying extracellular products across the blood-brain barrier. In studies of potential treatments for patients with amyotrophic lateral sclerosis (ALS), the researchers have demonstrated the safety of this strategy, with no significant adverse effects observed in 43 patients. Now, the researchers propose to use stem cells to deliver nerve protection into patients’ nervous systems before chemotherapy starts.

“We have recently discovered in the laboratory a compound that seems to be quite potent in protecting the nerve cell damage that cisplatin produces,” says Anthony J. Windebank, M.D., a consultant in Neurology at Mayo Clinic in Rochester, Minnesota. “We’re proposing to take stem cells from a patient’s adipose tissue (Figure) and use genome-editing technology to put this new compound into the stem cells. Then we would inject the stem cells into the patient’s cerebral spinal fluid, as a means of placing this compound into the central nervous system without its getting into the way of the cancer in the lungs, ovaries or breast.”

Figure. Stem cell therapy used in clinical trials at Mayo Clinic. A. Biopsy of adipose tissue is removed from a patient. B. Fat cells from the biopsy are placed in a culture dish. C. Stem cells (purple) are isolated from fat cells. D. Selected stem cells are re-engineered to include a nerve-protecting compound. E. Re-engineered stem cells are injected back into the patient. F. Re-engineered stem cells deliver the nerve-protecting compound into the central nervous system.
Re-engineering stem cells
Through the Mayo Clinic Center for Regenerative Medicine, Dr. Windebank has directed several studies involving the use of stem cells to promote nerve generation. “From our ALS studies, we know that these stem cells are very safe and last probably three to six months in the nervous system after injection,” he says. “Most chemotherapy is given over a three- to four-month period, so we hope the protective strategy for neuropathy would cover patients throughout their cancer treatments.”

In more than 20 years of research on chemotherapy-induced neuropathy, Dr. Windebank and colleagues have shed light on the mechanisms by which platinum drugs damage neurons. Dorsal root ganglion neurons are the primary target.

“Cisplatin actually causes the death of the neuron, not just nerve endings,” Dr. Windebank says. “Cisplatin neuropathy also has the unusual characteristic of continuing to worsen for two or three months after chemotherapy ends, which is why oncologists are very careful to cut back chemotherapy dosage when patients begin to develop neuropathy.”

Although chemotherapy-induced neuropathy can ease somewhat, most patients have residual symptoms years after cancer treatment ends. “Cancer treatment has improved phenomenally. The majority of people who have cancer are either cured or have long-term survival,” Dr. Windebank notes. “I see people who, 20 years after their cancer is cured, have almost forgotten they had it. But they’re still dealing with peripheral neuropathy that impairs their quality of life. By pursuing this strategy, we hope eventually to take cells that don’t normally get into the nervous system, give them some extra bullets with genetic engineering and then prevent chemotherapy-induced neuropathy.”

Applying Nanotechnology to Brain Tumors
Traditional cancer therapies target cells that are dividing rapidly or abnormally. However, those therapies don’t address the microenvironmental cues that play a key role in tumorigenesis. Nanotechnology (Figure 1) can provide tools to characterize and change that microenvironment, leading ultimately to the development of more effective therapy.

Researchers at Mayo Clinic in Jacksonville, Florida, are using nanotechnology applications to image the functioning of brain tumors within their microenvironments, as well as to deliver chemotherapy to tumors in the laboratory. These novel nanotechnology applications offer insight into tumor behavior and ultimately the prospect of treatment strategies tailored to an individual patient’s profile, for maximum clinical benefit.

“Nanotechnology allows us to study very complex interactions within a tumor simultaneously and in real time,” says Betty YS Kim, M.D., Ph.D., a consultant in Neurosurgery at Mayo Clinic’s campus in Florida. “As a result, we’re realizing it’s not just the tumor cells we should target. We need to recondition the environment and eliminate the factors that cancer cells need to grow in a patient.”

Windows on the brain
To image brain tumors in real time, researchers at Mayo Clinic’s campus in Florida developed a specialized multiphoton microscopy system. Nanomaterial dyes are used to target various types of cells within a tumor and its microenvironment. Laboratory mice are surgically implanted with tumor cells,
such as glioblastoma cells. The craniotomy site is closed with a transparent window-like structure. Once tumors develop, the mice are anesthetized and their brains imaged in real time.

“Our combination of nanomaterial dyes and traditional dyes can color-code different aspects of the microenvironment,” Dr. Kim says. “The microscope is so powerful that we can actually see single cells and even subcellular structures in a live animal.”

Various combinations of immune system modulators also are given to the mice. “We can capture in a video format how the tumor cells and other cells are moving in that environment,” Dr. Kim says. “Through this, we are exploring possible ways to enhance the immune system to fight off the cancer.”

The researchers have noted that within the tumor microenvironment, macrophages, microglia and T cells start to move toward the tumor as though ready to eliminate it. “We expect these immune cells to help clear the cancer cells,” Dr. Kim says. “But somehow, once getting close to the cancer cells within the tumor, cancer cells appear to be able to evade the immune cells as if they possess some kind of camouflage. We’re starting to understand what makes cancer cells invisible to immune cells, but it is really just the very tip of the iceberg in terms of understanding why.”

**A smart and stealthy foe**

Another major focus is investigating the use of nanoparticles to improve brain cancer treatment. In preliminary experiments, the Mayo Clinic researchers have embedded various chemotherapeutic agents within nanoparticles for delivery into tumors. “Once inside the tumor the nanoparticle starts to melt away and releases some of the chemotherapies,” Dr. Kim says. “The goal is to determine if this is a more effective method of delivery than oral chemotherapy.”

To further improve delivery of chemotherapeutics, the researchers are pursuing a strategy of improving tumor vasculature, in contrast to standard anti-angiogenesis therapies. Tumor vasculature, which tends to be porous and structurally heterogeneous, is a poor means of delivering medication. The Mayo Clinic researchers have worked to normalize tumor vasculature in lab mice. In a study published in *ACS Nano*, they found that tumor vessel remodeling enhances transvascular delivery of intermediate-sized nanoparticles, while smaller nanoparticles experience a significantly lesser degree of diffusional hindrance (Figure 2).

“After remodeling tumor vasculature, we have seen that nanomaterials no longer clump around the blood vessels but actually distribute more homogeneously inside the tumor,” Dr. Kim says. “We have shown that we can recondition the microenvironment.

“A brain tumor is not just a bunch of abnormal cells,” she adds. “It’s almost like a complex organism, stealthy and smart, with a group of supporting cells that work in concert to promote cancer cells to grow and evolve over time. Our goal is to turn these cells against the cancer cells or at least cut off their support.”

**For more information**


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**Figure 2.** Treatment with vascular endothelial growth factor receptor-2 (VEGFR2) antibody DC101 normalizes tumor vessels and improves intratumoral delivery and distribution of nanoparticles. A and B. Illustrations show improved uptake of nanoparticles (blue) post-treatment. C and D. Fluorescent microscopy demonstrates improved vasculature (green) and greater uptake of nanoparticles (red) post-treatment. Quantifying these effects showed that DC101 treatment resulted in increased total tumor area coverage by nanoparticles.
Research Highlights in Neurology and Neurosurgery

Radiosurgery Alone for Metastatic Brain Tumors
Approximately 30 percent of people with cancer develop brain metastases, and the incidence of these lesions is rising. Whole-brain radiotherapy (WBRT) significantly improves tumor control in the brain after stereotactic radiosurgery. However, WBRT is associated with cognitive decline. A multicenter trial led by researchers at Mayo Clinic in Rochester, Minnesota, has found that patients with three or fewer metastatic brain tumors treated with stereotactic radiosurgery had less deterioration in cognitive function and quality of life three months after treatment, and longer median survival, than patients who had stereotactic radiosurgery and WBRT. The researchers enrolled 213 patients between 2002 and 2013, and randomly assigned them to treatment with surgery alone or surgery followed by radiation. Study participants were evaluated for cognition and quality of life. Even patients given low-dose WBRT experienced worse long-term cognitive function than patients treated only with surgery. Patients treated only with stereotactic radiosurgery had higher quality of life than patients who had surgery and radiation therapy. Median overall survival was 10.4 months for patients treated with stereotactic radiosurgery alone and 7.4 months for patients treated with surgery and WBRT. The study findings suggest that, in the absence of improved overall survival, stereotactic radiosurgery alone might be a preferred treatment strategy for patients with one to three brain metastases. (Brown PD, et al. Effect of radiosurgery alone vs radiosurgery with whole brain radiation therapy on cognitive function in patients with 1 to 3 brain metastases: A randomized clinical trial. JAMA. 2016;316:401.)

Estrogen and Alzheimer’s Risk
Hormone therapy with conjugated equine estrogens (CEE) and medroxyprogesterone acetate, initiated later in menopause, has been found to increase the risk of dementia in women. Whether alternative formulations of hormone therapy can preserve neuronal integrity when administered early in menopause remains controversial. In a randomized, double-blinded, placebo-controlled trial, researchers at Mayo Clinic in Rochester, Minnesota, found that recently postmenopausal women who received CEE therapy had increased ventricular volumes — but without significant change in global cognitive function — compared with recently postmenopausal women who received a placebo. Study participants were ages 42 to 56, and had completed menopause within the previous five to 36 months. Participants were randomized to groups receiving oral CEE, transdermal estradiol-17 beta, or placebo pills for 48 months. MRI and cognitive testing were performed at baseline and at 18, 36 and 48 months. Changes in whole-brain, ventricular and white matter hyperintensity volumes, and in global cognitive function, were measured. Higher rates of ventricular expansion were observed in both the CEE and estradiol-17 beta groups compared with the placebo group; however, the difference was significant only in the CEE group. Rates of ventricular expansion correlated with rates of decrease in brain volume and with rates of increase in white matter hyperintensity volumes after adjusting for age. Changes were not different between the CEE and estradiol-17 beta groups for any of the MRI measures. The researchers suggest that further study with larger sample sizes and longer follow-up is needed. (Kantarci K, et al. Effects of hormone therapy on brain structure: A randomized controlled trial. Neurology. 2016;87:887.)

Parkinson’s Incidence May Be Increasing
Long-term trends in the incidence of Parkinson’s disease (PD) and parkinsonism have yet to be understood. In a population-based study, researchers at Mayo Clinic in Rochester, Minnesota, found a significant increase in PD and parkinsonism from 1976 to 2005, particularly among men ages 70 and older. Analyzing medical records of residents of Olmsted County, Minnesota, the researchers identified 906 patients who were diagnosed with parkinsonism, including 464 patients with PD, during the study’s 30-year time span. Age-adjusted incidence rates of parkinsonism were stable for women over that time frame. However, the age-adjusted incidence rates increased in men from 38.8 cases per 100,000 person-years between 1976 and 1985, to 56.0 cases per 100,000 person-years between 1996 and 2005. Similarly, the age-adjusted incidence rates of PD increased in men from 18.2 per 100,000 person-years between 1976 and 1985, to 30.5 between 1996 and 2005. Men ages 70 and older had a 24 percent higher risk of developing parkinsonism and a 35 percent higher risk of developing PD for every 10 calendar years spanned by the study. Although no trend was evident overall for women, women ages 70 or older experienced an increase in the incidence of PD with borderline statistical significance. The researchers caution that their results might merely reflect improvements in diagnosis of parkinsonism and PD. Further research is needed to replicate the findings and to identify possible lifestyle or environmental factors associated with the increased incidence. (Savica R, et al. Time trends in the incidence of Parkinson disease. JAMA Neurology. 2016;73:981.)

To read more about Mayo Clinic neurosciences research and patient care, visit http://www.mayoclinic.org /medical-professionals.
Expedited Patient Referrals to Mayo Clinic
Departments of Neurology and Neurologic Surgery

While Mayo Clinic welcomes appointment requests for all neurologic and neurosurgical conditions, patients with the following conditions are offered expedited appointments:

1. Cerebral aneurysms
2. Cerebral or spinal arteriovenous malformations
3. Brain, spinal cord or peripheral nerve tumors
4. Epilepsy with indications for surgery
5. Carotid disease