Charcot-Marie-Tooth Disease
In Focus

Streamlined diagnostic procedures, better data collection, a new clinical trials network and new laboratory research are the foundations of MDA’s CMT program

by Margaret Wahl

It begins with weakness in the muscles of the lower legs and feet, causing frequent tripping and ankle injuries. Feet are often so high-arched that comfortable shoes can’t be found.

Hands also can be affected, making it difficult to hold a pencil, type on a computer or play a musical instrument.

Loss of sensation in the lower legs, feet, hands and forearms often occurs. Although not as troublesome as weakness in these areas, it can make simple tasks more daunting.

The problem underlying all these signs and symptoms is Charcot-Marie-Tooth disease, or CMT, named after the three physicians who first described it late in the 19th century: Jean-Martin Charcot and Pierre Marie, two French neurologists, and British physician Howard Henry Tooth.

Symptoms generally begin in childhood or adolescence, although onset can be as early as infancy or as late as adulthood. The disease is usually slowly progressive, with the majority of patients able to function without severe disability — albeit with some difficulties — all their lives.

Often, CMT comes with a family history. When multiple family members are affected, the symptoms, though they’re not generally welcome, at least are recognized and understood.

When there’s no family history of CMT, children displaying symptoms initially may be warned to “stop dragging your feet” or “pay attention to what you’re doing,” or they may be treated for conditions they don’t have.

A problem in the peripheral nerves

CMT is a genetic problem in the peripheral nerves — bundles of fibers (also called axons) that run between the spinal cord and brain and the periphery of the body.

Peripheral nerves transmit signals from the brain and spinal cord out to the muscles, making movement possible; and back from the periphery of the body to the spinal cord and brain, allowing sensations to be perceived.

Since the early 1990s, defects that can cause CMT have been identified in more than 30 genes, improving diagnosis and understanding of the underlying molecular mechanisms.

The genes involved (many of which were identified by MDA-supported researchers) carry instructions for proteins that affect various aspects of peripheral-nerve function.

In most of the various types of CMT, the primary problem lies in one of three places:

- in the axon itself;
- in a sheath made of myelin (a mixture of proteins and fats) that surrounds each axon; or
- in the cells — known as Schwann cells — that lie along the surface of each axon and make this myelin sheath.

The myelin made by the Schwann cells winds around the axon the way paper towels wind around a cardboard tube. Among other functions, the myelin sheath insulates the axon and speeds nerve conduction along its length, just as insulation aids signal transmission through a wire.

If anything is amiss with the axon or its myelin sheath, motor and sensory signals can’t be effectively transmitted, especially over long distances such as between the spinal cord and the feet or hands.
As recently as the early 1990s, many experts hoped that understanding just a few genes that influenced the development or maintenance of myelin or axons (see graphic, left) would explain all of Charcot-Marie-Tooth disease. It didn’t turn out to be that simple, however. Today, there are dozens of genes recognized that, when flawed, can cause CMT.

Many CMT-related genetic mutations cause defects or abnormal levels of proteins in the myelin sheath. Other CMT mutations directly affect proteins in the axon. Still other flaws have complex, indirect effects on myelin, axons or the interactions between the two.

The currently used classification system for CMT is based on two criteria:

- whether it’s primarily the myelin or the axon that’s affected; and
- how the disease is inherited.

In broad strokes, CMT is divided into types 1, 2 and 4. (For complex reasons, type 3 no longer exists as a type of CMT.) Types 1, 2 and 4 are further divided into subtypes based on specific genetic mutations.

Severe forms of the diseases are sometimes called Dejerine-Sottas disease.

CMT types 1 and 2

*Type 1 are the problems where there’s abnormal myelin; in type 2, the myelin is...*
normal," says Michael Shy, a neurologist and professor of molecular medicine and genetics at Wayne State University in Detroit. Shy is a longtime MDA research grantee, a member of MDA's Medical Advisory Committee, and co-director of the MDA clinic at Wayne State.

If the myelin is damaged, nerve conduction velocity (conduction speed) slows down.

"You use nerve conduction velocities in the arm to see if they're slow or not," Shy says. "The cutoff, historically, has been 38 meters [about 125 feet] per second. If they're less than that, we say it's type 1. If they're more than 38 meters per second, we say it's type 2. For type 2, you usually see that the strength of the signal is reduced, but the main diagnostic criterion is speed of conduction."

Both CMT1 and CMT2 are inherited in an autosomal dominant manner, meaning it only takes one parent with one genetic defect to pass on the disease to a child. New mutations can occur spontaneously in a family with no previous history of the disease, after which the genetic mutation can be passed on to future generations.

"Type 1" and "type 2" still work as broad diagnostic headings, says Shy, but further subdivisions — types 1A, 1B, 1C, 2A, 2B, 2C and so forth — now indicate the specific genetic causes within each type.

The classification system gets a bit unwieldy, Shy explains, because it reflects a combination of causation (myelin versus axonal damage) and inheritance pattern.

**CMT type 4 and CMTX**

CMT disorders transmitted in an autosomal recessive pattern, meaning mutations must be inherited from both parents, are generally called type 4, whether they originate in the axon or the myelin, Shy says.

He notes, however, that this system of classifying recessive CMTs is not universal. Some systems use CMT4 to describe only myelin-related recessive CMTs.

CMT that's inherited in an X-linked pattern, meaning the gene flaw is on the X chromosome, usually manifest more severely in males than in females.

These originally were called "CMTX." Now, the most common form of X-linked CMT is called CMT1X, and there are at least four additional X-linked types.

For a full list of CMT subtypes, see Hereditary Motor-Sensory Neuropathies, on the Washington University Neuromuscular Disease Center site at http://neuromuscular.wustl.edu/time/hmsn.html.

**Flow charts help narrow down CMT types**

Today, the classification system, while still imperfect and unwieldy, provides physicians with a guideline for assigning the CMT type to a broad category, based on the results of nerve conduction velocity testing, physical examination, and patient and family history.

From there, if the family desires it, physicians can proceed to specific genetic testing, which is much more expensive, sometimes running into thousands of dollars.

With MDA support, Shy and his colleagues at Wayne State have developed a series of flow charts for physicians to follow that can drastically reduce the cost of CMT genetic testing by reducing the number of genes that need to be analyzed.

A paper on the subject was published online Jan. 28, 2011, in Annals of Neurology. (See http://onlinelibrary.wiley.com/doi/10.1002/ana.22166/pdf and also “Flow charts will aid CMT diagnosis” in Research Updates in the April-June 2011 Quest.)

The investigators found that the most common types of CMT, in order, were CMT1A, CMT1X, CMT1B and CMT2A.

Athena Diagnostics (athenadiagnostics.com), a commercial testing laboratory in Worcester, Mass., offers DNA analysis of some 15 CMT-related genes. Other laboratories that offer various types of CMT genetic testing can be found through Gene Tests (genetests.org), a listing overseen by the National Institutes of Health and sponsored by the University of Washington-Seattle.

Physicians and genetic counselors associated with MDA clinics can help locate and interpret genetic tests for CMT, as well as offer advice on how to focus testing to reduce costs.

**Why do genetic testing for CMT?**

When asked about genetic testing in CMT, Shy says, “This is the patient’s decision, not the doctor’s. There are pros and cons; it depends on the person.”

That said, he thinks there are some benefits to genetic testing beyond intellectual curiosity or research applications.

“Once you find the gene, you can get a handle on what the natural history of the disease might be and also the inheritance pattern,” Shy says.

For example, family histories may look the same for people with CMT due to a new, dominant mutation and for those with CMT caused by inheriting two recessive mutations, “but there’s a big
Providing a Network for Clinical Research in CMT

Individuals with CMT are encouraged to participate in a new international database

by Miriam Davidson

The field of Charcot-Marie-Tooth disease (CMT) research is expanding, and people with the disease can help move it forward.

Discovery of the complicated genetics underlying CMT has made it clear that more studies are needed to correlate the progression, symptoms and outward manifestations of the disease with its specific genetic type.

Researchers say that understanding the differences among the various types of CMT will improve the effectiveness of clinical trials and hasten the development of targeted treatments.

Knowing which mutation a person has also could help doctors better understand the variable nature of the disease, predict its likely course, and provide more specific information about medical management, inheritance patterns (for family planning), and other concerns.

With that aim in mind, MDA is partnering with other organizations in the United States and worldwide to establish a network of centers specializing in the diagnosis and treatment of CMT.

While training the next generation of CMT clinical researchers, the centers will collect and record genetic, biologic and other data from people with the disease.

“The goal is to make this an international network of the leading CMT centers where everybody evaluates patients the same way,” says Wayne State neurologist Michael Shy. Shy has received MDA support to establish a CMT clinical research network and registry (see “Sorting Out CMT,” page 2).

CMT database evolves into international network

The new CMT research network is an expansion of the CMT North American Database, which has been collecting information since 2001.

More than 800 people are enrolled in the CMT North American Database, which is housed at Indiana University and funded by MDA and the CMT Association.

The database contains information from people with CMT who were evaluated at these institutions:

• Wayne State University in Detroit
• Johns Hopkins University in Baltimore

Genetic testing now exists for many subtypes of CMT.

In Focus: Charcot-Marie-Tooth Disease

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As it turns out, MDA was working on its CMT database at the same time as the Office of Rare Diseases Research at the National Institutes of Health (NIH) was working to establish clinical research networks and specialized treatment centers for rare diseases.

One of the rare disease clinical research networks created by the NIH was for inherited neuropathies, the class of diseases to which CMT belongs.

In order to expand the network’s reach and enroll as many people as possible in its database, the North American CMT network is joining forces with the new NIH network.

The seven centers listed above all will be part of the Inherited Neuropathies Consortium Rare Disease Clinical Research Network (INC RDCRN). Additional sites joining or already in the consortium are:

- Washington University in St. Louis
- Vanderbilt University in Nashville, Tenn.
- University of Miami
- Center for Neuromuscular Diseases, National Hospital for Neurology and Neurosurgery, London, UK
- Great Ormond Street Hospital for Children, London
- Children’s Hospital Westmead, Sydney, Australia
- C. Besta Neurological Institute, Milan, Italy

“We hope the combined registry will provide an international resource for therapeutic trials, gene identification studies and natural history studies, even in the rare types of CMT,” Michael Shy says.

Through the participation of Annie Kennedy, MDA’s senior vice president for advocacy, MDA has taken a leadership role in the formation of the INC RDCRN.

“This has been an extraordinary partnership among MDA, Dr. Shy’s team and NIH’s innovative rare disease research network,” Kennedy says.

“It exemplifies how MDA strives to leverage funding with federal partners to accelerate research progress.”

**Participating in the INC RDCRN**

Researchers are asking anyone with CMT — whether or not they have been evaluated before — to come to one of the above-mentioned locations to be re-evaluated and have biological samples taken.

MDA will cover the evaluation and collection costs for people registered with the Association who visit an MDA clinic, but not transportation or other travel-related expenses.

Currently, three studies are under way through the INC RDCRN: a natural history study, a search for modifier genes for CMT1A (the most common form of CMT) and for new genes associated with CMT, and validation testing of the CMT pediatric score, a recently developed procedure for measuring CMT progression in children.

For more information on these studies, please go to rarediseasesnetwork.epi.usf.edu/ and click on the link for the Inherited Neuropathies Consortium.

Those wishing to stay abreast of developments in these CMT studies may log in to the RDCRN site and join the Patient Contact Registry.

People in the contact registry will receive updates regarding the Inherited Neuropathies Consortium, and may receive information about clinical trials in CMT that come out of this consortium. Those registered will be invited to participate in studies for which they qualify.

Alternatively, they can contact the specific sites for more information.

Readers who want to register or receive more information but who don’t have Internet access can call Lisa Rowe, neurology research assistant at Wayne State University, at (313) 577-1689.
Are myelin and axons ‘talking’ to each other — and what are they saying?

by Margaret Wahl

MDA-supported research in Charcot-Marie-Tooth disease is focused on figuring out what goes wrong at the molecular level in CMT-affected axons or the myelin sheaths that surround them, rather than on attempting to fix the problem directly or preserving nerve function in spite of it. A central theme emerging from the last decade of research is that myelin and axons require constant signals from each other to stay functional. Here is a look at work being done by three leading MDA-supported CMT researchers.

James Salzer: Keeping Schwann cells on track when myelin is lost

The axon and the myelin sheath need each other, says James Salzer, an MDA-supported professor of cell biology and neurology at New York University.

Myelin’s essential role in allowing for speedy conduction of nerve impulses and providing protection and insulation around axons is well-known, he says.

In addition, recent studies suggest that myelin provides sustaining signals to axons that help keep them intact.

Loss of the myelin sheath — demyelination — is first a problem for nerve conduction and eventually a problem for the health of the axon itself. The latter isn’t well understood and is an area of intense study, Salzer says.

But what interests him the most right now is another aspect of peripheral nerve function — namely, how the axon and the myelin sheath signal the myelin-making Schwann cells to either keep making myelin or to stop making it.

In many forms of CMT, Salzer suspects, the “make myelin” signals are disrupted, either because of abnormalities in the axon or in the myelin sheath. His lab is focused on trying to restore myelin production.

“If you don’t have an axon, the myelin sheath will break down,” Salzer says. “The current view is that, as the axon breaks down, it releases signals that tell the Schwann cell to break down its myelin sheath.” The Schwann cell seems to go back to a more primitive, undifferentiated state.

A similar process may happen if the myelin sheath is abnormal, as it is in many forms of CMT, including the most common form, CMT1A. It seems myelin proteins that are overproduced or abnormal can cause the same kind of shutdown in Schwann cells as occurs when the axon is defective.

The vast majority of CMT1A cases are caused by overproduction of a myelin protein called PMP22, because of the presence of an extra PMP22 gene. Only a small percentage of CMT1A cases are caused by a mutation in the PMP22 gene that causes an abnormal PMP22 protein to be made.

Salzer’s group is working on reducing production of PMP22 by targeting a molecular pathway known as mTOR.

“It’s a key nexus in the control of a process called protein translation,” he says, referring to how proteins are produced from the genetic material known as RNA. “That’s a path that I’m sure is going to become a robust area in CMT research over the next couple of years.”

But it’s not the only thing on his to-do list. “Rather than targeting the extra protein itself,” he says, “one could instead go to the consequences of the extra protein or the misfolded protein and target the signals that it induces.”

Stopping dedifferentiation signals and keeping Schwann cells differentiated, so that they’re in their myelin-making state, is an avenue Salzer plans to explore.
Like Salzer, Thien Nguyen has been thinking a lot about myelin and what it does for axons besides speeding up conduction.

Nguyen, a neurologist and neurophysiologist, is an assistant professor of neurology at Johns Hopkins University School of Medicine. He has MDA support to explore axonal protection as a strategy to treat CMT.

“Studies have shown that, even though there is slowing of the electrical signals when there is demyelination, patients actually do very well for many years,” Nguyen says. “But then eventually, after several years, the axons start dying, and that’s what leads to clinical [functional] deficits.”

Nguyen wanted to figure out how myelin protects and nourishes axons, as well as speeding nerve conduction.

He suspected there might be specific molecules in the myelin sheath that keep the axon alive, and that perhaps they could do so even when the sheath itself is unstable or improperly formed, as it is in type 1 CMT.

“The best place to look for such a molecule, we thought, is in the space between the myelin and the axon,” Nguyen says. And one of the most well-known molecules that sits there is myelin-associated glycoprotein, or MAG. Most of it is in the interface between the myelin and the axon, on the innermost coil of myelin.

“We figured that molecule would make perfect sense,” says Nguyen. “We asked, ‘If you lack this protein, is the axon more vulnerable to death?’ It turns out that yes, it is.”

More recently, Nguyen’s group has found that a protein called netrin 1 is located in the same myelin-axon interface as MAG and has many of the same functions. What’s particularly intriguing about netrin 1 is that it’s deficient in mice with a PMP22 mutation and a CMT1A-like disease.

“We think these myelin molecules — MAG, netrin 1 and possibly others — normally interact with axons,” Nguyen says, “and if that ability is interfered with, it will increase the vulnerability of the axon to injury and degeneration.

“Then we can extend that one additional step and ask, ‘If that’s the case, how about if we add them back? Are we able to reverse this process?’ That’s something we’ve been trying very hard to do.”

Meanwhile, he says, they’re also working on another approach — determining whether there’s a small piece of MAG and netrin 1 that’s the same and that sends protective signals to the axon. If that’s the case — and so far it seems to be — its small size would make it much more attractive as a candidate for drug development than a large protein would be.
Michael Granato, a professor of cell and developmental biology at the University of Pennsylvania, has MDA support to study degeneration and regeneration after damage to peripheral nerves in an animal that’s getting a lot of attention in scientific laboratories everywhere: the zebrafish.

Zebrafish have more in common with mammals, including humans, than most people think, Granato notes. But unlike other animals, they’re transparent, which is a huge advantage. Scientists can see what’s happening in structures like peripheral nerves by looking at them under a microscope while the fish is alive and swimming.

Granato says his research team has begun to revisit — with state-of-the art tools — many old assumptions about peripheral nerves and the cells with which they interact.

“Seeing is believing,” Granato says. “This not only pertains to what’s happening in the nerve that’s damaged, but also to other cell types. What’s happening to those? How do they interact with the peripheral nerves? This is really the basis of understanding what’s going on.”

Two cell types that have been studied in connection with peripheral-nerve degeneration and regeneration are the myelin-making Schwann cells and the macrophages, cells made by the immune system. The word “macrophage” means “big eater,” and these cells gobble up debris from degenerating tissue in many different circumstances.

It’s long been assumed that macrophages go out to the damaged nerve some time after the damage has occurred, he says. But ongoing work in his lab studying nerve damage in the see-through zebrafish is focusing on the question of whether macrophages arrive even before the fibers start to break down.

Granato’s group is also using genetic tools to see how taking away macrophages would affect nerve regeneration in the zebrafish. If macrophage-supplied cleanup efforts had a positive effect on regeneration, attracting more macrophages to the site of an injury could be a therapeutic avenue. But if those efforts made matters worse, perhaps inhibiting macrophage recruitment would help.

Granato now has zebrafish with a mutation in the GARS gene, the cause of type 2D CMT. He’s testing the idea that nerve regeneration after injury is inhibited in these fish, and he believes figuring out the underlying molecular signals could ultimately be important for understanding and possibly treating some types of CMT.

Looking at something in real time in a model like the zebrafish “looks somewhat different” from what is seen in biopsy samples, he says. “We’re finding a lot of things that we..."