



UNTANGLING THE COMPLEXITY OF DYSTONIA

40+ YEARS OF RESEARCH ACHIEVEMENTS



RESEARCH PROGRESS: THEN & NOW

40+ YEARS

STIMULATING RESEARCH PROGRESS WORLDWIDE

In 1976, DMRF launched a global research effort toward a cure.

DMRF has funded critical scientific advancements while investing over decades to support a research environment in which investigators can dedicate careers to dystonia.

Advancements in basic and clinical research have led to sophisticated genetic studies, insights from neuroimaging technologies, and unprecedented drug discovery efforts.

40 YEARS AGO

Only 200 accounts of dystonia had ever been published.

A small group of pioneering neurologists linked dystonia to a cluster of nerves in the brain called the basal ganglia.

Patients were routinely misdiagnosed and treated for psychiatric illness.

The field of “movement disorders” did not yet exist.

There were no accepted treatments.

The US Food & Drug Administration had not yet approved the use of botulinum neurotoxin to treat dystonia.

Children with severe generalized dystonia were treated with ablation brain surgery.

No genetic changes were associated with dystonia. The genetics of dystonia was not understood.

Pioneering researchers were just beginning to map the motor circuitry of the brain. Deep brain stimulation was not applied to treat dystonia.

Brain imaging techniques such as magnetic resonance imaging (MRI) and computed tomography (CT) were coming into use.

Little was known about proteins involved in dystonia. There were few potential drug targets.

Lack of knowledge prevented dystonia dedicated drug development.

TODAY

US National Library of Medicine alone catalogues 20,000 studies and reviews on dystonia. Medical articles pertaining to dystonia are published daily.

Origins of dystonia in the brain appear to lie in the complex interactions of the basal ganglia and cerebellum in conjunction with the cortex. Dystonia is widely understood as a circuitry disorder.

Dystonia is defined as a neurological syndrome of involuntary muscle contractions affecting one or more sites of the body, frequently causing twisting and repetitive movements or abnormal postures.

Dystonia is considered more than a movement disorder. Non-motor aspects appear to include differences in sensory processing, depression, and anxiety.

Comprehensive treatment strategies have been published and widely accepted.

Botulinum neurotoxin injections are a treatment of choice for dystonia. Four brands are available.

Treatment options for children and adults may include oral medications, botulinum neurotoxin injections, deep brain stimulation, and supportive approaches such as physical therapy.

Numerous genes and gene markers are identified. Close relationships among various genes and their proteins are being explored.

Deep brain stimulation (DBS) was approved by the FDA in 2003 under a humanitarian device exemption for the treatment of chronic, intractable primary dystonia including generalized, segmental, hemidystonia, and cervical dystonia. DBS is used on a case-by-case basis for a widening spectrum of patients including childhood and adult onset generalized dystonia, tardive dystonias, cervical dystonia, myoclonus-dystonia, and others.

Technological advancements in brain imaging, brain stimulation methods, and surgical practices have dramatically improved. Non-invasive neuromodulation therapies are being explored that seek to replicate the benefits of deep brain stimulation without neurosurgery.

Every dystonia gene discovery points to a protein that may be a potential drug target. For instance, DYT1 gene discovery led to a cellular protein in the brain called TorsinA, which causes dystonia when abnormal due to genetic mutation. The cell-level role and behavior of TorsinA and other dystonia proteins are being clarified.

In addition to existing treatments, investigations are identifying new drugs to treat dystonia.



The Dystonia Medical Research Foundation's (DMRF) research efforts have amassed a wealth of data, across multiple scientific disciplines, which together are creating a layered and sophisticated understanding of dystonia.

TABLE OF CONTENTS

CLINICAL QUESTIONS	6
NEUROPHYSIOLOGY	8
DISEASE MODELS	10
GENES & MOLECULAR BIOLOGY	12
DRUG DISCOVERY	16
CLINICAL TRIALS	17
UNIQUE DYSTONIAS	18
MOVING FORWARD	19

DMRF—UNTANGLING THE COMPLEXITY OF DYSTONIA

40+ Years of Research Achievements

On our way to finding a cure for dystonia, we must try to understand its causes and biological mechanisms. This is a complex task. There are many forms of dystonia—some caused by well-defined gene mutations, while some fall outside genetic classification and might be caused by brain network abnormalities of yet unknown origin. While this complexity can be considered an obstacle in revealing the nature of dystonia, it can also, oddly enough, offer many strategic advantages in our struggle to understand this disease.

The Dystonia Medical Research Foundation's (DMRF) research efforts have amassed a wealth of data, across multiple scientific disciplines, which together are creating a layered and sophisticated understanding of dystonia.

Multiple dystonia-causing gene mutations and alterations have been discovered, yet our increasing understanding of brain circuits and networks allowed us to redefine dystonia as a complex network disorder. As we progress in research on all fronts, we combine our knowledge from genetics and molecular and cell biology with what we have learned from human and animal brain studies. This current necessity drives DMRF's science efforts and funding strategies. Our funding portfolio reflects this approach.

Simple Questions that are Not So Simple

DMRF has been supporting dystonia research for more than 40 years. Advancing dystonia science takes more than providing funds to researchers. It requires:

- ④ Gathering people who are or might be genuinely interested in understanding dystonia and researching its biological underpinnings.
- ④ Continuous activity to educate doctors, researchers, the general public, and decision-makers about dystonia, to inspire more research and create more funding opportunities.
- ④ Continuous monitoring of the field, organizing critical research meetings (small and large), seeking novel outlooks, and taking chances.
- ④ Engaging the patient community in supporting research efforts, through participation and advocacy.

Of course, there are different approaches to do all that, from more passive, i.e. waiting for the field to mature and then asking questions, to very proactive, where careful assessment of the field allows for bold initiatives to be undertaken. Over the years DMRF has done it all. Has it paid off? How do we

measure scientific progress? How do we know we are on the right track? What matters is the scientific progress that leads to furthering our knowledge about dystonia and allows us to achieve novel treatments.

Scientific research is a time-dependent process. Knowledge accumulates, gets evaluated, transforms, changes, and never stays 'the same.' What was a mystery yesterday might be obvious today and may fundamentally change tomorrow.

Peer-reviewed publications remain the best measure of productivity and accomplishments of research goals. Published data are tangible, shareable and, perhaps most importantly, inspire new questions and investigations.

The following summary highlights achievements by multiple investigators supported by DMRF. Their original research produced a mountain of data. Not every hypothesis turned out to be true, not every attempt resulted in a successful conclusion, but *all of this work contributed to a greater understanding of dystonia*. Such is the nature of research. It started by asking simple questions.

CLINICAL QUESTIONS

The purpose of DMRF's research efforts is to improve the lives of individuals affected by dystonia. This means translating research discoveries into medical advancements relevant to diagnosis, treatment, and overall care. Asking fundamental questions about dystonia is essential for better understanding the disorder as well as understanding impact on patients.

What is dystonia?

Dystonias are very diverse disorders, both clinically and by cause. Identifying the manifestations and causes of dystonia are critical to research progress. Before physicians can diagnose and treat dystonia, they must understand what it is—and what it is not.

Since the mid 1970s, DMRF has been the driving force behind developing a consensus definition and classification of the dystonias. This effort, based on DMRF-sponsored workshops and recently led by Dr. Alberto Albanese, culminated in the publication in 2013 of a paper redefining dystonia and offering a novel classification system based on clinical features and etiology that has been widely accepted by neurologists all over the world.

The revised classification system both reflects the complexity of dystonia while streamlining the language used to describe and discuss it. It is a tool available to clinicians and researchers everywhere.

As of 2020, the paper has been cited by more than 1,000 publications on dystonia.

How many people are affected by dystonia? Is it rare?

These questions are not purely academic but have an impact on government-provided funding as well as pharmaceutical and medical device industry efforts in assessing the size of potential target populations and markets. Patients and families rightly want to know how many others like them are out in the world. DMRF recognized this quite early and sponsored studies on dystonia epidemiology.

One of the studies, led by Dr. Caroline Tanner, formed a basis for an extensive National Institutes of Health (NIH) study conducted over several years. Although we are still far from establishing precise numbers for dystonia

A critical contribution of DMRF to the dystonia field is a history of supporting preliminary studies that inspire future investigation by independent researchers, often subsequently receiving robust federal funding.

prevalence and incidence worldwide and in the US, these studies inspired others in countries around the globe to explore dystonia epidemiology in well-defined populations where clinical information is accessible through national health care services.

Dystonia is certainly not as rare as we thought. However, individual genetic forms can be extremely rare.

Why there are so many forms of dystonia?

When DMRF began its operation, dystonia was considered a set of symptoms, a poorly defined disease entity. Pioneering clinical studies by Drs. Stanley Fahn and C. David Marsden, both supported by DMRF, led to a more precise description of dystonia, recognition of its origins, and even first therapies. Their efforts solidified, for example, that adult onset focal dystonias and inherited childhood onset dystonias were indeed variations of the same disease. A very complex picture of dystonia as a set of disorders finally emerged.

DMRF's early support of several movement disorders clinical centers specializing in dystonia in the United States, Canada, and United Kingdom formed the basis for genetic studies that later allowed for identification of novel dystonias and the gene mutations that cause them. The DMRF-funded



clinical centers were pioneering hubs for dystonia patient care and treatment, including early use of botulinum neurotoxin injections and anticholinergic medications.

The centers established standards of diagnosis, clinical testing, and treatment of different dystonias.

Dystonia is not a psychiatric disorder, but does it have psychiatric components?

Pioneering work by Drs. Stanley Fahn and David Marsden redefined dystonia as a neurological movement disorder, eradicating the mischaracterization and stigma of dystonia being purely psychiatric disorder. Yet, with time, it was clear that there are psychiatric components—whether primary (as part of the disease symptomatology) or secondary (as an effect of living with dystonia).

Early DMRF-supported work by Dr. Rachel Saunders-Pullman shed light on the importance of addressing psychiatric issues in dystonia. This approach has been recently continued by Dr. Mateusz Zurowski in application to various forms of dystonia. DMRF-funded projects awarded to Drs. Kathryn Peall and Marina de Koning-Tijssen concentrate on complex neuropsychiatric problems in myoclonus-dystonia.

Addressing these issues is critically important for patients who seek to understand the origins of their psychological symptoms, especially considering that many medications have side effects directly affecting mood and behavior.

Psychological and psychiatric symptoms can be as disabling, even more so, as dystonic movements and postures. Understanding and evaluation of psychiatric symptoms in dystonia patients is critical to comprehensive treatment to improve quality of life.

NEUROPHYSIOLOGY

Dystonia is a disorder that has the potential to solve many puzzles about the brain. Efforts to better understand dystonia are often fundamentally rooted in discerning a better understanding of how the brain generates and controls body movement—which is relevant to virtually everyone in the world. Understanding brain function is among the most challenging and exciting frontiers of medicine.

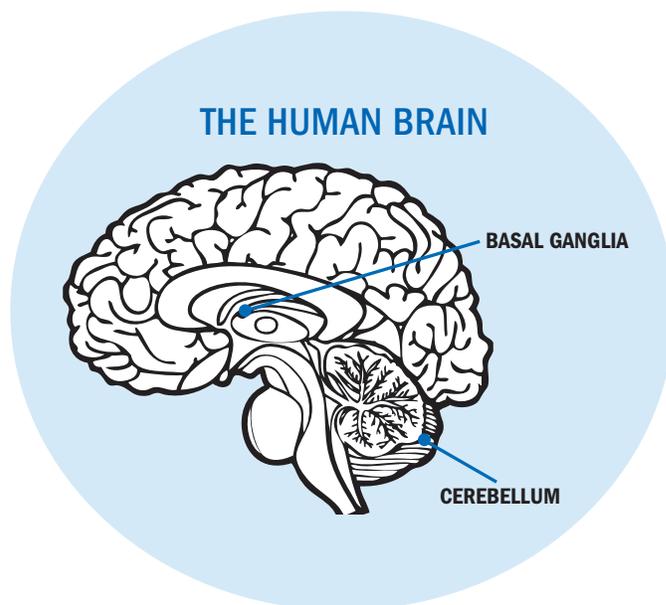
What is different about the dystonia brain?

From simple neurophysiology to circuits, networks, plasticity, and deep brain stimulation (DBS), DMRF-supported investigators have made remarkable progress in understanding the underlying neurology of dystonia.

Pioneering work by DMRF Scientific Director Emeritus Dr. Mahlon DeLong clearly pointed to the brain circuit abnormalities in dystonia. The basal ganglia have been the focus of dystonia research for decades now.

From simple neurophysiology to circuits, networks, plasticity, and deep brain stimulation (DBS), DMRF-supported investigators have made remarkable progress in understanding the underlying neurology of dystonia.

New directions have also emerged encompassing neuronal physiology, other brain circuits and networks, mechanism of plasticity and the basis of deep brain stimulation so successfully applied to dystonia. The list of DMRF-supported researchers in this area is quite long and includes (in alphabetical order): Drs. Robert Chen, Joseph Classen, N. Charles Harata, Ellen Hess, Leighton Hinkley, William Hutchison, Daniel Leventhal, Sabine Meunier, Simon Overduin, Antonio Pisani, Robert Raike, Angelika Richter, John Rothwell, Vikram Shakottai, Roy Sillitoe, Philip Starr, and their collaborators.



Collectively, these investigators performed hundreds of experiments to explore the mechanisms that might be involved in dystonia:

- ④ They established a role for specific neurons in the striatum (part of the basal ganglia).
- ④ Mapped the interactions and relationships between different neurotransmitters that influence movement control in dystonia.
- ④ Identified novel circuits and proteins that are pivotal in movement control.
- ④ Redefined the role of the cerebellum as a major part of complex brain networks affected in dystonia.
- ④ Extensive studies in dystonic patients revealed intricate changes in brain neurophysiology recorded during movement or induced by invasive and noninvasive brain stimulation.

What is emerging from these studies is a very complex picture of dystonia as a brain disorder involving specific brain pathways where interplay of dedicated neurons drive movement in dystonia. Such neuronal circuit aberrations are anchored in cell and protein machinery where neurotransmitters dictate outcomes of movement control.



With every study in this area more questions arise and it is expected that higher level analyses will be required to construct a model of brain function in dystonia, which would be a pivotal milestone in the field.

Can we see the dystonia brain in action?

Brain imaging techniques have significantly contributed to our understanding of the network and circuit abnormalities that determine symptoms of dystonia. Recognizing the importance of visualizing changes in the living human brain, DMRF has been funding neuroimaging research for the last 30 years.

Dystonia brain imaging remains one of the most important research areas, and is now used by numerous DMRF grantees as an analytical and diagnostic technique. It is expected that continuously developing imaging technologies will allow

for even more precise mapping of networks and circuits in dystonia to better define its neural mechanisms and reveal potential biomarkers.

Trailblazing work by Drs. David Eidelberg, Maren Carbon, Anne Blood, and others provided great advances in identifying and mapping functional and structural changes in dystonia. Imaging detected white matter changes and allowed for detection and characterization of functional connectivity changes. In DYT1 dystonia, manifesting and non-manifesting mutation carriers showed distinctive changes when compared to normal individuals. Recent work by Dr. An Vo adds another dimension to these studies: complex computational analyses provide unprecedented insight into the inner working of dystonic brains.

DISEASE MODELS

Disease models replicate specific aspects of human dystonia in highly controlled environments, allowing investigators unique opportunities to advance our understanding. Disease models are essential to progress because they enable researchers to ask questions and conduct investigations that are impossible to pursue in humans.

What can we learn about dystonia from rodents, fish, worms, and flies?

Many disease-related studies rely on animal models that reproduce major aspects of disease. Since dystonia is a neurological disease manifesting as a very complex movement disorder, it has been extremely difficult to reproduce human-like symptoms in animals. Discoveries in dystonia genetics created new opportunities to develop animal models carrying disease-causing mutations.

- ④ Drs. Yuqing Li, Bill Dauer, and Rose Goodchild developed a series of dystonia transgenic animals, some carrying human gene mutations causing DYT1, DYT6, or DYT11 dystonia or having corresponding genes deleted. In a series of papers the authors extensively characterized the mice in terms of their motor behavior and brain abnormalities. These animal models have been shared with others and studies utilizing them have generated numerous publications, deepening our knowledge about biological mechanisms of genetic dystonias. These animals have become invaluable in studying neurophysiological and cellular mechanism of dystonia.
- ④ Drs. Guy and Kim Caldwell used a worm, *C. elegans*, to model dystonia virtually in a dish. Their studies on torsinA biology in worms opened up new opportunities for quick and convenient modeling of dystonia. More importantly, they were able to use the worms to screen for torsinA modifying factors and drugs. This resulted in the development of a human clinical trial for one of the most effective drugs in the screen.
- ④ Dr. Naoto Ito had been working with fruit flies for years. Since the flies have only one torsinA-related gene, he removed the gene and observed that the insects started to move abnormally. More importantly, there was a decrease in brain dopamine levels and reduction in the activity of a key enzyme in the dopamine pathway.

Expansion of this observation and a series of genetic experiments established the fly as a valid dystonia model.

- ④ Others have attempted developing models of dystonia, primarily based on the DYT1 mutation, reasoning that the severity of this form might help in seeing motor symptoms in animals. Dr. Kathrin Grundmann succeeded in creating the largest of all, a transgenic DYT1 rat, hoping that the size of the animal would facilitate neurophysiological and anatomical studies. The DYT1 rat is currently used in a number of investigations providing a unique insight into movement control in dystonia.
- ④ A few DMRF-supported researchers provided further evidence that animal models of different kinds can be very useful in deciphering dystonia mysteries. Dr. Lesilee Rose added new levels of sophistication to study dystonia and torsinA in worms, and Dr. Edward Burton developed a zebrafish model of DYT1 dystonia.

All these invaluable and necessary studies place dystonia among the best studied neurological diseases, with ethically developed animal models proving to be essential tools for investigating dystonia mechanisms and drug testing.

Can we study dystonia by using iPSCs?

Modeling diseases is not limited to animal models. Induced pluripotent stem cells (iPSCs) are adult cells, provided from consenting volunteers, that have been genetically reprogrammed to an embryonic stem cell–like state and can be transformed into different cell types. The advent of iPSCs provides unprecedented opportunities to analyze cellular processes that happen in patient-derived cells that can be ‘converted’ into neurons—something absolutely critical for dystonia research. DMRF had realized these opportunities quite early and sponsored several studies in this direction.

All these invaluable and necessary studies place dystonia among the best studied neurological diseases.



Drs. Nutan Sharma and Cris Bragg derived a number of cells that could be transformed into neurons and successfully studied. This included cells obtained from carriers of the DYT1 dystonia-causing mutation. These cells, which have been deposited in cell banks and are available to other researchers, are an invaluable tool for studying internal cell pathways as well as in testing potential new drugs.

Similarly, Dr. Anne Grünewald generated a number of iPSC lines for myoclonus-dystonia. These cells are currently being utilized in another study, sponsored by DMRF and led by Dr. Karen Grütz, collaborating with an international team to understand the protein and gene networks that drive the disease process.

Such studies place dystonia research at the forefront of modern cell biology.

GENES & MOLECULAR BIOLOGY

Molecular biology is the study of life and disease at the molecular level. Investigating dystonia from this perspective is a deep dive into the inner workings of cells, proteins, DNA, and genes—and the nature of the dysfunction present in these systems when dystonia strikes.

Visionary funding decisions made by DMRF paid off, and the DYT1 gene discovery marked the beginning of a new era in dystonia research.

What causes hereditary dystonia?

Hereditary dystonias offer unique opportunities for understanding fundamental aspects of the disorder, and therefore are an essential research focus.

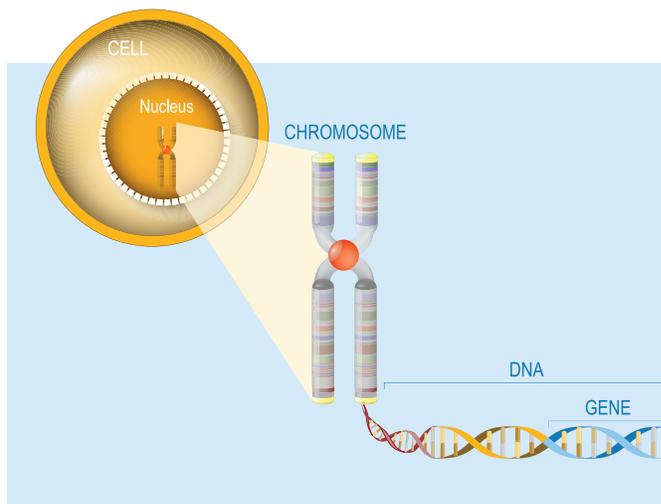
Every gene discovered to cause dystonia offers the opportunity to understand the disease mechanism; we can observe the genetic changes that initiate the disease process and have the opportunity to track the cellular and biological processes those genetic changes influence. Investigators are finding commonalities among genes, distilling the complexity of dystonia genetics into convergent pathways.

Furthermore, dystonia-causing genes do not express in every carrier. The reduced penetrance of dystonia-causing genes suggests that there may be protective factors that prevent gene expression in some people, or vulnerabilities that predispose dystonia in others.

DMRF-funded investigators are responsible for numerous critical genetic advancements:

Q DYT1/TOR1A

The 1997 discovery of the first early-onset isolated dystonia gene, DYT1, by Drs. Laurie Ozelius, Xandra Breakefield, Susan Bressman, and their co-workers was a culmination of many years of collaborative efforts by neurogeneticists and clinical neurologists. Visionary funding decisions made by DMRF 10



years earlier paid off, and the discovery marked the beginning of a new era in dystonia research.

DYT1 dystonia has become the most studied form of hereditary dystonia. Its molecular underpinnings have been gradually revealed by a long series of studies in large part initiated and supported by DMRF. Combined clinical and genetic diagnosis redefines this disease and helps in making treatment decisions. Understanding the precise genetic cause of DYT1 dystonia has allowed for preimplantation genetic diagnosis and *in vitro* fertilization in cases when one of the prospective parents carries the pathogenic mutation—this has ended a painful legacy of dystonia in multiple families. Finally, the DYT1 gene discovery inspired and motivated other neurogeneticists to identify gene mutations causing other forms of hereditary dystonia.

Q DYT6/THAPI

A team effort, over several years, shed light on what causes a very rare and unusual form of dystonia. Clinicians and geneticists led by Dr. Laurie Ozelius and supported by DMRF finally identified the culprit in 2006. Since then the THAPI gene has become a focus of intense studies by Drs. Laurie Ozelius, Susan Bressman, Michelle Ehrlich, Tatiana Fuchs, William Dauer, Pedro Gonzalez-Alegre and



others, that continue to help us understand this form of severe dystonia but also shed light on other genetic dystonias.

Mutations in THAPI have revealed that diverse dystonia genes disrupt similar neuronal pathways and functions. THAPI mutation-induced dysfunction of certain cellular pathways in DYT1 dystonia may represent a point of convergence in the pathophysiology of several forms of inherited dystonia. Several DMRF grants and fellowships have enabled such studies and helped researchers to obtain sizeable grants from National Institutes of Health (NIH).

Q DYT25/GNAL

One of the most spectacular genetic findings was discovery of DYT25 dystonia-causing gene abnormalities. An international research group led, again, by Dr. Laurie Ozelius in 2013 found pathogenic mutations in a gene coding for a GNAL protein

that, unlike torsinA at the time of its discovery, had been well-known and extensively studied.

What is more, the protein occupies a strategic position in the cell linking many proteins involved in cell signaling. This opens up new, currently explored, possibilities to target this and neighboring proteins with pharmacological agents.

Q DYT16/PRKRA

Work by one of the earliest DMRF research fellows, Dr. Andrew Singleton, provided proof that mutations in the stress-response gene PRKRA cause a dystonia-parkinsonism syndrome.

This link between cellular stress response and dystonia continues to intrigue researchers and brought to the field renowned PRKRA experts like Dr. Rekha Patel whose DMRF

Continued on page 14

Understanding torsinA's role in the secretory pathway led to molecular screens that have been used to assess drug candidates.

and National Institutes of Health (NIH) supported research linked the mechanism of this form of dystonia to other genetic forms where cellular stress response disturbance seem to be involved.

In the never-ending quest to understand hereditary dystonias, many DMRF-supported researchers have been deciphering the genetic background of extremely rare dystonias as well as attempting to find gene mutations in more common, perceived as non-hereditary, dystonias. Overall, these ongoing studies demonstrate multifactorial causes of dystonias.

With the growing number of causative and predisposing genes identified we begin to realize that there are converging mechanisms that one day may explain why dystonia develops.

Understanding such mechanisms may eventually help in designing successful treatment strategies.

TorsinA—what does it do in the cell? How does the dystonia-causing mutation affect its function?

The protein torsinA was identified in 1997 once the DYT1 gene, causing early onset torsion dystonia, was discovered by DMRF-funded investigators Drs. Laurie Ozelius, Xandra Breakefield, Susan Bressman, and their collaborators. This previously unknown protein was quickly recognized as belonging to the AAA+ ATPase family known to play many roles in the cell.

DMRF has funded a number of investigators to find out more about torsinA in its normal state and the dystonia-causing mutant. This new protein attracted the attention of several leading experts in biochemistry and molecular and cell biology.

Critical milestones have been reached as a result of creative work by numerous researchers supported by DMRF over many years including (in alphabetical order): Drs. Xandra

Breakefield, Cris Bragg, Jeff Brodsky, William Dauer, Pedro Gonzalez-Alegre, Rose Goodchild, Gregg Gundersen, Christoph Kamm, Phyllis Hanson, John Ladas, Brett Luring, Patrick Lusk, Gant Luxton, Flavia Nery, Gordon Rule, Christian Schlieker, Thomas Schwartz, and their collaborators.

The major achievements include:

- ④ Establishing a role for torsinA in nuclear envelope development and formation.
- ④ Uncovering torsinA involvement in the secretory pathway and synaptic vesicle machinery.
- ④ Characterizing aberrant effects of the DYT1 dystonia-causing mutation that include morphological and functional changes.

A separate line of research identified a number of novel and well-known proteins that interact with torsinA:

🔍 LULLI & LAPI

Proteins like LULLI and LAPI are required for torsinA enzymatic activity – something that had been very difficult to demonstrate until these proteins were identified as necessary to activate torsinA.

🔍 BIP

A protein called BiP, a molecular chaperone that interacts with torsinA in a well-defined cellular transport mechanism called the secretory pathway, has been found to play multiple roles in the biogenesis of torsinA. TorsinA itself has been shown to be a chaperone as well.

Detailed understanding of the role torsinA plays in the secretory pathway led to designs of molecular screens that have been used to screen drug candidates.

A series of studies in animal and cell models revealed that a loss of torsinA function, caused by a pathogenic mutation, leads to abnormal formations in brain cells and can cause neuronal death. Such effects occur only during the window

of time when symptoms develop pointing to the developmental aspects of DYT1 dystonia, whereby symptom onset occurs almost always in mid-childhood.

More recently, an unexpected role for torsinA as a regulator of lipid metabolism has been discovered. This discovery may have implications for possible treatment strategies for DYT1 dystonia.

One of the most momentous achievements to date was obtaining a high resolution protein structure of torsinA by Dr. Thomas Schwartz and colleagues. After many years of efforts, this success allows for deeper understating of torsinA functional state and its higher-level architecture.

More and more studies point to specific cellular pathways commonly shared among different dystonias.

Resolution of the three-dimensional structure opens many possibilities for fundamental research on torsinA and for rational drug design to fit drug molecules directly into the torsinA protein.

Several of these studies have been facilitated by a program initiated by DMRF with a biotech company, described in the drug testing/screening section below.

Dystonia genes do not act alone—should we target malfunctioning cellular pathways?

The multiplicity of dystonia clinical forms combined with an ever-growing number of dystonia-causing genes has made it daunting for researchers to propose unified theories of dystonia mechanism—can such a heterogeneous group of disorders share a single common mechanism?

However, years of research inspired by genetic discoveries has begun to yield results. More and more studies point to very specific cellular pathways commonly shared among different dystonias. A concept once treated as outlandish becomes a surprising reality.

Again, DMRF provided long-term support for such, initially risky, studies. Dr. Antonio Pisani approached the problem using neurophysiology and discovering that different forms of genetic dystonia have the same neural abnormality that involves complex interaction between ‘classical’ receptors involved in inter-neuronal communication. His studies set the gold standard for assessing dystonia pathology at the neuronal and brain levels.

Drs. Nicole Calakos, Pedro Gonzalez-Alegre, Cris Bragg, and Mark LeDoux independently contributed to developing the idea that dystonia might be a molecular/cellular network disease, in which changes at the neuronal level impact various brain structures that control movement. DMRF continues to support these researchers who have received substantial awards from the National Institutes of Health (NIH) and Department of Defense (DOD).

These studies benefited from a DMRF-led contractual project in which a biotechnology company, BioFocus, screened their proprietary inhibitory RNA library in a cell model based on Dr. Xandra Breakefield’s research. In these cells, torsinA was genetically removed and close to 5,000 genes tested for torsinA restoration of function. Such selected genes and proteins they code would point to pathways affected in DYT1 dystonia that could be used as potential therapeutic targets.

Ultimately, 355 unique gene/proteins targets were identified, many of them pointing to pathways later identified by researchers with whom the results were shared. This information has been shared with various researchers helping them to select or identify novel proteins, genes, or molecular pathways that are of critical importance for DYT1 dystonia. More directly, these results, to be published soon by Dr. Cris Bragg and collaborators, helped identify a novel pathway implicated in DYT1 dystonia.

DRUG DISCOVERY

Because the dystonia community cannot necessarily wait for pharmaceutical and biotech companies to invest in dystonia on their own, drug discovery and development efforts are an important part of DMRF's science strategy. These efforts run simultaneous to DMRF's ongoing efforts to attract and engage industry partners.

What drugs and compounds should we be testing?

Before testing a drug candidate in humans, researchers need to carry out hundreds of experiments that ensure the drug is not toxic, that it targets the right protein or gene, that it can be easily tested in a dish or in animals, and that it shows signs of efficacy in animal models.

Such studies are often a result of many years of meticulous research, and sometimes are prompted by a chance opportunity. Obviously, they are risky. Many, if not most, fail to deliver.

DMRF prides itself as a pioneer and trailblazer in dystonia research and as an organization charged with delivering new and better treatments for dystonia patients. Not surprisingly, DMRF has been funding drug testing research for years. Some of that research included development of novel methods for drug screening.

- ④ Drs. Guy and Kim Caldwell used their *C. elegans* worm model to screen a collection of drugs already approved by the Food & Drug Administration (FDA). A selected few were further modified and rescreened. Surprisingly, ampicillin, a well-known antibiotic, showed some promise. An ampicillin clinical trial for DYT1 dystonia motor symptoms is still underway.
- ④ Dr. Cris Bragg and colleagues developed a series of molecular screens for DYT1 dystonia based on torsinA trafficking in the cell. The group synthesized a large number of chemical compounds that could potentially correct torsinA mutation-related deficits.
- ④ Drs. Zixiu Xiang and Jeffrey Conn tested and studied their own anticholinergic compounds. These studies led to development of drug candidates that are under further development with support from National Institutes of Health (NIH) and Department of Defense (DoD), where dystonia is one of the targeted diseases in the Congressionally Directed Peer-Reviewed Medical Research Program .
- ④ Drs. Pedro Gonzalez-Alegre and Yuqing Li independently pursued opportunities to 'silence' the DYT1 mutation by using RNA molecules. The molecules showed great promise in cell culture, yet appeared to be toxic to animals. Despite these setbacks, a pharma company expressed interest in potentially developing similar drugs.
- ④ Another company, AstraZeneca, offered a drug-in-development to test in dystonia models. DMRF supported Drs. Antonio Pisani and David Standaert to do that. The drug, a nicotinic receptor agent, did not show positive effects, however, its action indicates that this type of drug has potential in dystonia treatment.



CLINICAL TRIALS

The DMRF has invested substantial effort in preparations to quickly begin clinical trials when a new dystonia treatment needs testing. Creating this infrastructure to support clinical trials is as critical to dystonia research progress as the discovery of novel treatments to test.



Clinical trials—who needs them?

Deeper understanding of dystonia inevitably leads to designs of new treatments. Pharmacological agents, medical devices, and other therapeutic approaches must be tested in humans. Clinical studies in dystonia patients and regular clinical trials provide the ultimate answers about treatment safety and efficacy. There is no evidence-based medicine without properly conducted clinical trials.

DMRF has been working with the Dystonia Coalition, Dystonia Study Group, National Institutes of Health (NIH), academic clinicians, and statisticians as well as pharma companies on dystonia clinical trial readiness. This resulted in a joint publication in 2013 on ‘Designing Clinical Trials for Dystonia.’

There is no evidence-based medicine without properly conducted clinical trials.

In addition, the Foundation had already sponsored several clinical trials in the past led by Drs. Susan Fox (dronabinol) and Steven Frucht (limb immobilization), among others. Although these pioneering studies have not led to commercial drug/treatment development, they raised considerable interest from pharmaceutical and device companies and set the stage for larger studies that we hope to conduct in the future. DMRF continues to collaborate with patient groups for clinical trial readiness, for example via the Global Dystonia Registry.

UNIQUE DYSTONIAS

Focused research efforts into specific types of dystonia simultaneously expedite discoveries relevant to affected individuals and help clarify questions or concepts about dystonia more broadly.

What do we know about myoclonus-dystonia?

In a distinct research program, supported by the Brown Family Foundation, DMRF has been supporting projects aimed at studying this very rare form of dystonia with unusual myoclonic features. Due to its rarity, myoclonus-dystonia (M-D) research is primarily limited to a few neurological centers in Europe and North America. A series of dedicated workshops set the agenda for this program.

Achievements include the following:

- ④ A major effort from Dr. Kathryn Peall, helped by M-D researchers around the world, is establishing the first ever registry of M-D patients. The registry will be linked to a biorepository being set up by Dr. Mark LeDoux. Systematic collection of clinical information and patient derived DNA, cells and tissue samples will facilitate more systematic studies on M-D.
- ④ Several research themes have been supported by the program through grants and contracts. Drs. Christine Klein, Anne Grünewald, and Karen Grütz developed and currently study induced pluripotent stem cells derived from M-D patients' biosamples (fibroblasts). These cells constitute a unique model of M-D where genetic and cellular effects of gene mutations causing M-D can be studied.
- ④ Drs. Kathryn Peall and Marina de Koning-Tijssen tackle a difficult issue of psychiatric symptoms in M-D. These are pioneering studies in this field pointing to inherent psychiatric comorbidities in M-D patients and implications for quality of life.
- ④ Drs. Yulia Worbe, Emmanuel Roze, and Marie Vidailhet pursue an innovative line of research using methodological advances in the dynamic imaging of brain activities during task performance or movement anticipation.

- ④ Dr. Marie Vidailhet, a leading deep brain stimulation (DBS) researcher, is studying the effects and determinants of DBS in M-D with the goal to optimize and harmonize DBS protocols to directly benefit M-D patients treated at different centers around the world.

The vision of the Brown Family Foundation for this program and DMRF's efforts to implement it into research practice cannot be underestimated in an orphan and neglected rare disease like M-D.

What do we know about musician's dystonia?

Musician's dystonia is another form of quite peculiar dystonia affecting skilled musicians. It is both enigmatic in its causes and personally devastating for professional musicians. Musician's dystonia provides a striking model for the task-specificity of dystonia symptoms—a phenomenon often seen in non-musicians, too, and not entirely understood.

DMRF has been passionate in supporting research in this area. One of the biggest mysteries is its genetic background. No causative genes has been discovered so far, yet a genetic predisposition is strongly suspected. Dr. Eckart Altenmüller and his collaborators found that there are a few genes that might exactly do that—predispose over-practicing musicians to dystonia.

In ongoing studies, others try to discover brain abnormalities in musicians and ways to correct them. Dr. Robert Chen uses his expertise to modulate brain connections in the cerebellum of musicians. Dr. Christine Kim takes a more direct approach and studies hand movements using a unique instrumentation that measures hand forces in string players. The hope is to use this knowledge in devising rehabilitation and prevention strategies for musicians.



What's next?

DMRF-supported investigators continue to wrestle the challenges of studying dystonia into opportunities for innovative problem-solving and ambitious discovery. We are not simply amassing new results but comparing and connecting these discoveries and observations to generate a deeper understanding of dystonia as a complex brain network disorder.

Investigations are ongoing to:

- ④ Understand the role of common dystonia cell-molecular pathways to clarify the biological basis for dystonia and design new therapies with the broadest potential for multiple types of dystonia.
- ④ Continue clarifying dystonia-associated brain pathways and networks for the purpose of developing novel treatment strategies and targets.

- ④ Further clarify the function and role of torsinA, and strategies for targeting the protein for therapeutic intervention.
- ④ Identify dystonia biomarkers in the brain.
- ④ Develop innovative treatments from vibration therapies to rehabilitation therapy to machine-guided neuromodulation.
- ④ Gain insight into the mechanism of deep brain stimulation.
- ④ Collaborate on multidisciplinary partnerships and projects.

As the results from these studies appear in the medical literature, the dystonia field will continue to develop a greater understanding of the disorder and increasing clarity in how to best care for those affected. The DMRF will continue to support and stimulate the dystonia field and partner with researchers across the globe on behalf of patients with all types of dystonia and their families.



One East Wacker Drive, Suite 1730

Chicago, Illinois 60601-1980

PHONE 312 755 0198 • 800 377 DYST (3978)

WEB www.dystonia-foundation.org

EMAIL dystonia@dystonia-foundation.org

