

**TRANSLATIONAL RESEARCH IN PRIMARY MITOCHONDRIAL DISEASES:
CHALLENGES AND OPPORTUNITIES March 8-9, 2012 • NHGRI Conference Area •
Rockville, Maryland**

EXECUTIVE SUMMARY AND WHITE PAPER

Chairs:

Vernon Anderson (NIGMS) and Carlos T. Moraes (University of Miami)

Organizers:

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Sponsors

Office of Rare Diseases Research (ORDR-NCATS) and United Mitochondrial Disease Foundation (UMDF).

EXECUTIVE SUMMARY

On March 8-9, 2012 the NIH intramural and extramural research communities as well as representatives from industries and foundations with a common interest in primary mitochondrial diseases met at the NHGRI conference center to identify the major barriers to the development of better treatment for mitochondrial diseases. Besides the importance to the patient population, it has become clear in the last decade that advances in understanding and treating primary mitochondrial diseases will impact research into a large number of degenerative conditions known to have a significant mitochondrial dysfunction component in their pathogenic mechanisms (secondary mitochondrial diseases) that affect millions of people, including: Alzheimer's disease, Parkinson's disease, diabetes, ALS, autism spectrum disorders, and many others.

The main goals of this workshop were: (1) Share information related to primary mitochondrial disease among the NIH Intramural and Extramural Research Program Investigators, (2) Develop and/or enhance systems to facilitate future collaboration and sharing of information, (3) Survey obstacles, needs and priorities of primary mitochondrial diseases research, (4) Develop mechanisms to enhance translation of basic science discoveries to diagnostics and therapeutics.

Definition of Primary Mitochondrial Diseases

Mitochondrial function plays a central role in multiple cellular functions, including oxidative phosphorylation, lipid metabolism, citric acid cycle, urea cycle, metal groups biosynthesis, among many others. Therefore, determining the boundaries of Primary Mitochondrial Diseases can be challenging. However, traditionally the term Primary Mitochondrial Diseases has been used to describe diseases associated with defects in oxidative phosphorylation function attributable to genetic differences in either mitochondrial or nuclear DNA.

Identified Barriers and Recommendations

1) As effective treatments for mitochondrial diseases do not exist, it is important to continue to invest in the basic biology and physiology of mitochondria. As an example, the lack of effective techniques to manipulate the mtDNA has been a major barrier to the development of treatments for an important group of patients.

2) Stable long-term patient registry and biospecimen repositories are essential to the development of clinical trials. This resource requires collaboration between NIH and patient advocacy groups to provide funding and promote patient enrollment.

3) With sufficient numbers of patients in the registry, natural history trials, which are essentially non-existent at the moment, must be initiated. These are critical to plan randomized clinical trials, which will be required to test new drugs and procedures.

4) NIH study sections need to be knowledgeable in mitochondrial diseases and how defects in mitochondrial physiology impact organ function and disease phenotypes.

5) Drug development will require better models, better lead molecules and better non-invasive endpoints for evaluating the effectiveness in trials. The application of innovative high throughput screens for molecules that can improve impaired mitochondrial function has the potential to generate new lead compounds. Development of new imaging modalities and identification of biomarkers of mitochondrial function can lead to standardized methods of gauging the effectiveness of potential therapeutics in clinical trials. Finally, animal models will continue to reveal the role of mitochondrial function in the etiology of mitochondrial disease and provide a platform to test potential therapeutic agents.

6) Next generation sequencing methods of mtDNA and known nuclear mitochondrial genes will contribute to the diagnosis of primary mitochondrial disease. Pooling data from multiple sites, including universities, industries and international, will improve the ability to identify rare deleterious variations.

7) A primary mitochondrial disease website should be developed that identifies the resources available to researchers, clinicians and the general public interested in mitochondrial disease. Continuing training of physicians in Primary Mitochondrial Diseases is also a critical part of this effort.

8) Establish a working group comprised of willing chairs and co-chairs and other volunteers from the mitochondrial community. This committee will be charged with developing a system for continued interaction among NIH Institutes and with the extramural community. The working group would determine the overall goals and specific objectives to be achieved through the continued interaction and identify the mechanisms that would be required. This system will help ensure that the recommendations of the Workshop are addressed and that other opportunities derived from coordination/sharing of information are pursued.

A more detailed description of the discussions can be found in the accompanying white paper, particularly in the summary of session 7, which expands on the barriers and opportunities identified.

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The oxidative phosphorylation (OxPhos) system

The OxPhos system is composed by five multimeric enzyme complexes (complex I-V) that are embedded in the inner mitochondrial membrane. To be assembled and fully active, these complexes need not only their structural subunits, but also hundreds of ancillary proteins that promote assembly and function. As an example, the enzyme COX10 adds a lipid (farnesyl) to a heme prosthetic group forming heme type a, an essential part of the catalytic center of complex IV. *In vivo*, some of these complexes are associated into even larger structures known as supercomplexes or respirasomes. Therefore, defects in a large number of genes can lead to Primary Mitochondrial Diseases (i.e. with primary OxPhos defects). This definition does not minimize the impact of diseases associated with secondary defects, which are much more common. Nonetheless, by making advances in the treatment of genetically defined Primary Mitochondrial Diseases, the scientific and medical community will be able to apply the lessons learned to more common but complex disorders affecting mitochondrial function.

Why the focus on Primary Mitochondrial Diseases?

Although relatively rare as single phenotypic entities, Primary Mitochondrial Diseases are now recognized as a major health issue by most physicians with a prevalence of approximately 1-5,000, although the identification of new genes that affect OxPhos is leading to an appreciation that Primary Mitochondrial Diseases are more common than previously realized. These devastating diseases are complex, with highly variable presentations and without effective treatment. Therefore, a concerted effort is required to address this public health need. In addition, the study of this group of disorders has and continues to yield a wealth of information in the basic biology of mitochondria, including the identification of novel genes of previously unknown function. Moreover, mitochondrial function plays a central role in degenerative diseases and aging, making the understanding of this organelle important not only to patients with Primary Mitochondrial Diseases but also to patients with more common disorders, such as diabetes, Parkinson's disease and age-related hearing loss among many others. These factors led us to propose that the study of Primary Mitochondrial Diseases provides a window to the understanding and treatment of many conditions afflicting our aging population. The workshop program was developed with a focus on identifying the opportunities for, and barriers to, treating these diseases. Ample time was left for discussions during the sessions and at the end of the workshop. Below are the summaries of the scheduled sessions.

Format of the workshop

To address challenges and opportunities in primary mitochondrial diseases, the workshop was organized with a distinct translational focus. However, the importance of basic research was also emphasized, as, with very rare exceptions, no effective therapies to these disorders are currently available. The organizing group invited the participation of a broad segment of the research community and industry. The following seven sessions were organized by expert co-chairs from both the NIH intramural and extramural/academic institutions. Importantly, each session included ample time for discussion, which proved to be valuable.

Session 1: Mitochondrial Biology and Mitochondrial Medicine: Where Are We in 2012?

Co-Chairs: Robert S. Balaban, Ph.D. (NHLBI), and Vamsi Mootha, M.D. Harvard Medical School

Session 2: Expanding Clinical Research Capabilities

Co-Chairs Lynne Wolfe (NHGRI) and Chuck Hoppel (Case Western Reserve University)

Session 3: Emerging Therapeutic Approaches – Sharing Resources and Opportunities for Therapeutic Development

Co-Chairs: William Gahl, M.D., Ph.D. (NHGRI) and Douglas Wallace, Ph.D. (Children's Hospital of Philadelphia)

Session 4: Product Development Activities of the Industry – Lessons Learned

Co-Chairs: Danuta Krotoski, Ph.D. (NICHD) and Gregory Enns, M.D. (Stanford University)

Session 5: Opportunities and the Need for Research Collaborations and Information Sharing Among Research Partners

Co-Chairs: Petra Kaufmann, M.D., M.Sc. (NINDS) and Michio Hirano, M.D. (Columbia University)

Session 6: Barriers and Opportunities to the Development and Use of New and Emerging Basic Research and Clinical Technologies

Co-Chairs: Steven Zullo, Ph.D. (NIBIB), Maren Laughlin, Ph.D. (NIDDK) and Carmen Mannella, Ph.D. (Wadsworth Center)

Session 7: General Discussion and Recommendations

Chairs: Carlos Moraes and Vernon Anderson.

An open discussion about the different topics addressed during the workshop was conducted.

This paper highlights the identified challenges and describes the discussions and exchanges originated during these sessions. It also makes recommendations that could be useful to NIH Institutes with interest in developing or increasing their portfolio in Primary Mitochondrial Diseases. The session summaries, written by the respective co-chairs and edited by the conference chairs, describe the essence of the topics covered.

Session 1: Mitochondrial Biology and Mitochondrial Medicine: Where Are We in 2012?

Co-Chairs: Robert S. Balaban, Ph.D. (NHLBI), and Vamsi Mootha, M.D. Harvard Medical School

Dr. Robert Balaban (NHLBI) described a series of studies on the basic biology of mitochondria performed at the NHLBI laboratories, emphasizing how the mitochondrial proteome differs between organs and tissues. Moreover, protein modifications expand the variability of the functional mitochondrial proteome.

Dr. Vamsi Mootha emphasized why it is important to study Primary Mitochondrial Diseases by citing the following: Mitochondrial diseases are common inborn errors, estimated to be ~1/4000-5000 live births. There are clear links to common diseases, including L2HGDH and gliomas; PARK/PINK and Parkinson's disease; mtDNA A3243G and diabetes. Studies on mitochondrial diseases also provide insights into basic biology, although genotypic and phenotypic variability make Primary Mitochondrial Diseases extremely challenging to diagnose and treat. He described a new approach to identify mutations causing mitochondrial diseases, the mito-exome, which combines exome sequencing and

comparison with previously catalogued genes coding for mitochondrial proteins. Dr. Mootha also emphasized the value of case discussions involving a diverse group of experts, from clinicians to biochemists and geneticists. He concluded that:

- The study of primary mitochondrial diseases constitutes a special opportunity for the NIH.
- Primary mitochondrial disease is an ideal application area for personal genomics.
- New technologies are needed to measure mitochondrial activity *in vivo*.
- The need for fundamental studies of mitochondria to understand pathogenesis cannot be overstated.
- Pharma/biotech are developing an interest in primary mitochondrial diseases.
- National centers and collaborative networks will be required to overcome the challenges posed by these disorders to realize curative therapies.

Dr. Douglas Wallace emphasized how the study of “primary mitochondrial diseases” has the potential of illuminating the genetics and pathobiology of a wide range of common metabolic and degenerative diseases as well as cancer and aging. He made the point that the reason that mitochondrial function has been largely overlooked in Western medicine is that mitochondrial biology pertains to energy while Western medicine has traditionally classified diseases based on anatomy. In the West, diseases are defined by the organ that is most significantly affected based on the assumption that organ-specific symptoms are due to organ-specific defects. The manner in which the NIH Institutes are organized around specific organ systems is an example.

However, the study of primary mitochondrial disease has taught us that systemic energy defects can result in organ-specific symptoms and that the symptoms that can be seen in primary mitochondrial diseases encompass all of those that are manifest by the common metabolic and degenerative diseases. In addition, Western medicine has assumed that the genes that are important for human health are encoded by the nuclear DNA and inherited according to the Laws of Mendel. However, the most important energy genes are coded by the cytoplasmic mitochondrial DNA (mtDNA) and to assemble a functional cellular bioenergetic system requires a combination of one to two thousand nuclear DNA genes as well as thousands of copies of the mtDNA genes. Therefore, the study of primary mitochondrial diseases has taught us that the anatomical and Mendelian emphasis of Western medicine has overlooked the importance of energetics, yet energy flow through our bodies is the vital force that is central to our lives and health.

Dr. George Santangelo, Director of the NIH Office of Portfolio Analysis gave an objective overview of the NIH efforts in mitochondrial research. Using a keyword search, it was found that NIH is currently investing \$609 million in mitochondrial research as follows: Primary mitochondrial diseases - \$18 M, Non-primary mitochondrial diseases - \$165 M, Basic mitochondrial research - \$393 M, Secondary use of mitochondrial information - \$ 33 M. Approximately \$125 M have been awarded to projects with a translational, therapeutic, or diagnostic component. Figures 1 and 2 illustrate the distribution of these investments.

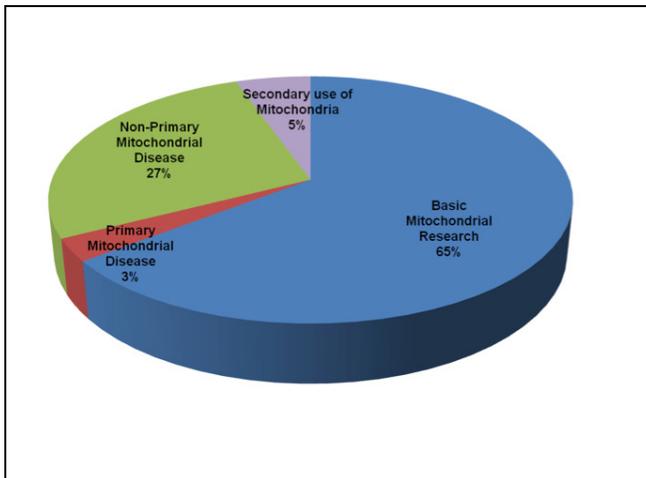


Figure 1. Distribution of NIH investment on different aspects of mitochondrial research.

Topics	Funding in Millions	Percentage of Total
Total Funding in Mitochondrial Research	\$609	
Primary Mitochondrial Disease	\$18	3%
Translational	\$5	
Therapeutic/Diagnostic	\$8	
Other	\$5	
Non-Primary Mitochondrial Disease	\$165	27%
Translational	\$67	
Therapeutic/Diagnostic	\$45	
Other	\$53	
Basic Mitochondrial Research	\$393	65%
Secondary Use of Mitochondria	\$33	5%

Figure 2. Distribution of NIH investment on different aspects of mitochondrial research, with a breakdown on clinical aspects.

The definition of “Primary Mitochondrial Diseases” used in this analysis was broad, including defects in fatty acid metabolism and iron homeostasis. Figure 3 lists the main disease entities included.

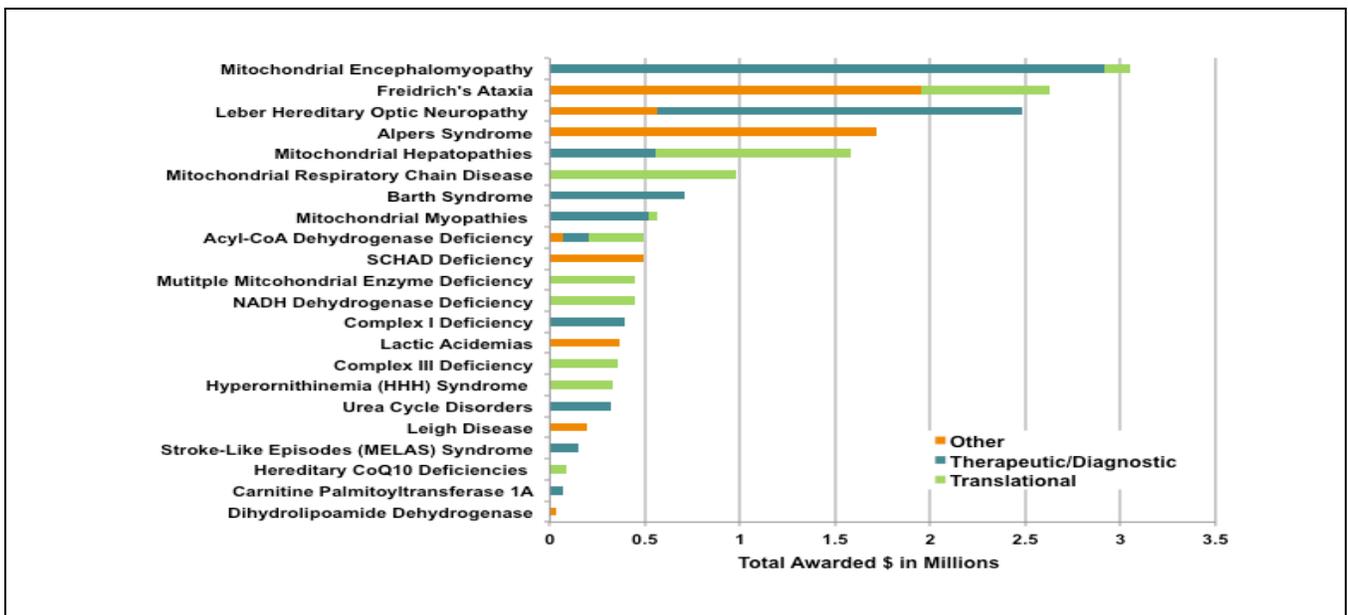


Figure 3. Mitochondrial Diseases included in the “Primary Mitochondrial Diseases” search in the NIH portfolio.

Research in Primary Mitochondrial Diseases was part of the portfolio of several NIH institutes, but it was concentrated mostly at NIDDK, NINDS, NICHD and NEI (Figure 4).

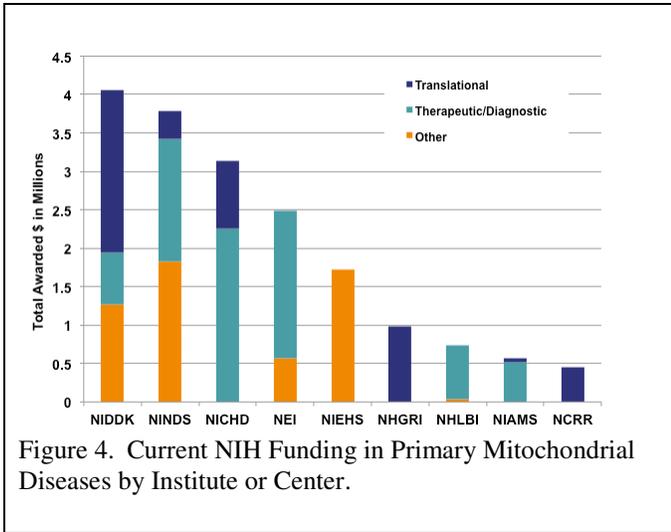


Figure 4. Current NIH Funding in Primary Mitochondrial Diseases by Institute or Center.

Funding of secondary mitochondrial diseases reflects the broad interest of the scientific community in mitochondrial dysfunction. Several neurological disorders have been associated with mitochondrial defects (Figure 5). This interest was also observed for non-neurological conditions. In fact, NIDDK has the largest portfolio on research on secondary mitochondrial defects (Figure 6).

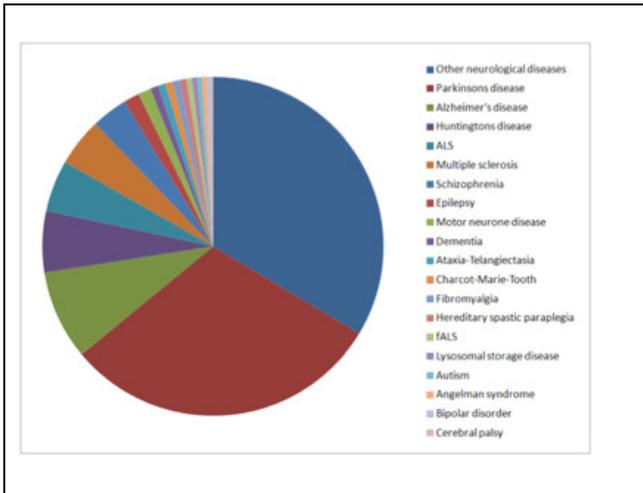


Figure 5. Breakdown of Neurological Disease Category: \$49 Million

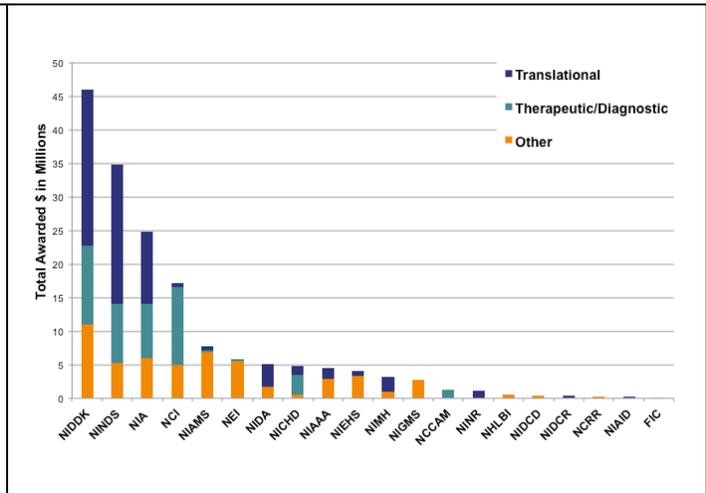


Figure 6. Current NIH Funding in Non-Primary Mitochondrial Diseases by NIH Institute or Center.

Basic research in mitochondrial function is funded at a higher level. Although this effort is funded by different Institutes, NHLBI has by far the largest portfolio (approximately \$170M), followed by NIGMS (approximately \$55M), NIDDK (approximately \$39M), and the NIA (approximately \$38M).

Session 2: Expanding Clinical Research Capabilities (Co-Chairs Lynne Wolfe NHGRI and Chuck Hoppel Case Western)

Dr. Alexandra Sanford, who has been intimately associated with the North American Mitochondrial Disease Consortium (NAMDC), described the efforts of NAMDC to register patients. She emphasized that patients/families want therapies approved and are looking to participate in trials.

To achieve this goal we would need a strong database for mitochondrial diseases that would help set the stage for tracking natural history, which are lacking at the moment. It would also provide a forum for standardization of mitochondrial diagnosis and support patient and researcher access for developing clinical research.

The North American Mitochondrial Disease Consortium (NAMDC), now funded by the NICHD and the United Mitochondrial Disease Foundation (UMDF), has started this effort. The short-term goal is 500 patients by the end of year 3 and a long-term goal of enrolling 100% of diagnosed patients in the registry. It also plans to have all NAMDC patients participating in at least one study, whereas all NAMDC patients would be part of natural history studies.

However, there are barriers to achieve these goals, including: The current culture of medicine and patient care, the fact that different sites have different IRB requirements, regulatory issues, the existence of multiple Registries (RDCRN, NAMDC, GRDR) and finally, the need for designated Research Assistants for consenting, data collection and data entry.

Dr. Peter Stacpoole (University of Florida) described his experience with Primary Mitochondrial Disease clinical trials and made suggestions that could improve the planning and execution of clinical trials for mitochondrial diseases. He noted that the focus of mitochondrial research is heavily skewed, as randomized controlled trials (RCT) of primary mitochondrial diseases are much rarer than the number of diseases described.

Through 2010, RCTs of Primary Mitochondrial Diseases have been performed mostly in single centers. Six trials reported positive treatment effect on ≥ 1 clinical or biochemical primary endpoints. No industry funding sources were acknowledged, reflecting low interest from commercial companies. However, this trend is clearly changing, as defects in mitochondrial function are taking center stage in more common diseases.

The poor history of mitochondrial diseases RCT is also reflected by the fact that there are no known pre-trial meetings with FDA and/or Health Canada. Likewise, there are no known new drug applications filed. Some of the barriers to developing RCTs for Primary Mitochondrial Diseases include an historical aversion to RCTs by mitochondrial disease lay and professional communities, which is related to inadequate investigator training. In addition, inexperienced and uninformed reviewers about special challenges of rare disease trials may not recognize the relevance of these studies. It is also worth mentioning that funding and regulatory agencies with contrasting philosophies, timetables and communication channels pose a challenge to inspiring investigators interested in RCTs.

To address these barriers, UMDF and NAMDC are necessary and critical, but are not sufficient. It is important to continue the education of lay and professional communities. The development of a guide perhaps through the ORDR could provide one-stop shopping for a comprehensive approach to writing

first-class RCT applications and review them appropriately. A key element would be across agency timelines for submission.

The creation of standing Rare Disease Study Section with trained reviewers chosen through a registry and managed by ORDR could coordinate proposal requirements and funding cycles to promote cost-sharing. A stand-alone FDA regulatory division also with trained reviewers sensitive to investigator funding timelines and limited budgets should be in place as well. As per the request from the NIH representatives, UMDF has submitted a list of over 70 qualified reviewers for consideration.

Dr. Charles Hoppel (Case Western Reserve University) addressed the challenges of developing standardized clinical endpoints for mitochondrial diseases, a group of relatively heterogeneous disorders. Multiple clinicians see patients with clinical presentations believed to be consistent with a mitochondrial phenotype, who have abnormal integrated mitochondrial function, but normal ETC analysis of muscle and mitochondria. Poor insurance reimbursement for advanced mitochondrial diagnostics inhibits our ability to clarify these diagnoses or identify new mechanisms of mitochondrial diseases. There is a compelling need to support innovative strategies that will identify new causes of mitochondrial diseases; carrier protein defects, assembly factors deficiencies, gene-gene interactions etc. Clearer diagnostic criteria especially for tissue analysis (e.g. Use of electron microscopy), proteomic and metabolomics methods as well as interpretation of novel molecular findings need to be developed.

Dr. Sihoun Hahn (Seattle/Transgenomics) described some of the difficulties related to making diagnosis with new technologies as well as the progress made through partnering with industry and Pharma, that address diagnostic challenges such as the significant overlap among many genetic conditions, variable test results from diagnostic laboratories assaying muscle electron transport chain enzymology especially when it could be secondary to other medical conditions, ~400 genes have been implicated in mitochondrial disease, and ~1500 estimated proteins in mitochondria. The use of Next Generation Sequencing as a diagnostic tool for mitochondrial diseases should avoid invasive, lengthy and often inconclusive tests as well as unnecessary treatment, while simultaneously promoting the development of new therapies, allow accurate genetic counseling, assess prognosis and potential therapies. Next-generation sequencing is a promising technology for clinical diagnosis of mitochondrial diseases as many patients with diagnosed/suspected mitochondrial disease may not be the direct result of disorders of energy production making the analysis of respiratory chain complex genes alone insufficient to diagnose these patients. Additionally, the genetic heterogeneity of mitochondrial diseases appears higher than suspected and may even include multi-gene involvement to explain complex clinical presentations.

Session 3: Emerging Therapeutic Approaches – Sharing Resources and Opportunities for Therapeutic Development

Co-Chairs: William Gahl, M.D., Ph.D. (NHGRI) and Douglas Wallace, Ph.D. (Children's Hospital of Philadelphia)

The session began with discussion of the programs and resources available through NIH Institutes and Centers. Dr. John Gallin, director of the NIH Clinical Research Center, described the Clinical Center, its GMP drug facility, MRI center, PET facility, clinical research training courses, and Bedside-to-Bench Award Program, which is now dominated by extramural-intramural collaborative efforts. He announced a new RFI to solicit input on partnerships that will allow extramural investigators to use the

Clinical Center, in collaboration with an intramural investigator. An RFA will be announced soon, with grants amounting to as much as \$500k per year, renewable.

Dr. John McKew of the NIH Center for Translational Therapeutics (National Center for Advancing Translational Sciences) described the Therapeutics for Rare and Neglected Diseases (TRND) Program, designed to bring intramural and extramural labs together for preclinical drug development. TRND provides in-kind research rather than monetary grants. Dr. McKew also discussed Bridging Interventional Development Gaps (BrIDGs), which provides access to contract services for GMP manufacturing, formulation, pharmacokinetic testing, animal toxicology, provision of clinical trial supplies, and advice in preparing Investigational New Drug (IND) applications. To use the facility, the PI must have a lead agent, and eventually prepares the IND. Thirty of 180 applications have been approved to date.

Dr. William Gahl, Clinical Director of NHGRI, described the NIH Undiagnosed Diseases Program and gave examples of patients with possible mitochondrial diseases. He noted the imminent start-up of a clinical trial of EPI-743 for the treatment of patients whose fibroblasts in culture respond to this drug with increased resistance to oxidative stress.

Dr. Elizabeth McNeil of NINDS described Institute initiatives testing promising drugs in Phase 2 clinical trials via a Clinical Coordinating Center at Mass General and a Data Coordinating Center at the University of Iowa. NeuroNEXT reviews proposals and a protocol working group is established for accepted proposals. Clinical sites are scattered at medical centers across the country; the sites can accept or decline participation in any NeuroNEXT protocol. Master trial agreements are established, and a central IRB reviews the protocol. Application is through U01, small business, or X01 mechanisms. Intramural investigators can collaborate with an extramural investigator, and natural history studies are suitable. The next deadlines are August 2 and December 2, 2012.

Dr. Renee Wong of the Division of Cardiovascular Sciences of NHLBI described RFA-HL-10-002, “The Role of Cardiomyocyte Mitochondria in Heart Disease: An Integrated Approach”, as one example of a mitochondria-related initiative. Another is PAR-11-307, “Discovery of Genetic Basis of Mendelian or Monogenic Heart, Lung, and Blood Disorders.” In addition, NHLBI sponsors Proteomic Centers throughout the country, and a SMARTT (Science Moving Toward Research Translation and Therapy) Program, provides IND directed services.

Dr. Danuta Krotoski, of the Office of the Director of NICHD, listed offices and divisions that might be pertinent to mitochondrial disease investigators. These include the Intellectual and Developmental Disabilities Research Centers, the Newborn Screening Translational Research Network, the NICHD Brain and Tissue Bank for Developmental Disorders, the Medical Rehabilitation Research Infrastructure Network, and the Rare Diseases Clinical Research Network, and the Pediatric Pharmacology Research Units Network. She also mentioned NICHD initiatives relevant to primary mitochondrial diseases, largely R01 programs.

Dr. Yong Yao, of the Division of Neuroscience and Basic Behavioral Science on the NIMH described opportunities for High-Throughput Screening (HTS) for the discovery of chemical probes. More than 370,000 compounds are available for HTS today. There are several “core centers” where NIH-funded HTS efforts are underway, including: **Comprehensive Centers:** Broad Institute- biochemical, cell-based, HCS assays, automated microscopy, small molecule microarray, BSL-2. NIH Chemical Genomics Center (NCGC) – qHTS, enzyme, protein-protein, BSL-2 assays, HCS. Sanford/Burnham

Institute - biochemical, cell-based, HCS assays; NMR based ligand optimization, PK/ADME. Scripps Institute – GPCRs, protein-protein, enzyme, ion channel, reporter assays; PK/ADME.

Specialized screening centers: Johns Hopkins University – ion channels, yeast two-hybrid assays; automated patch clamp. University of New Mexico (UNM) – HT flow cytometry; real time kinetic analysis, cell/bead based multiplex assays. Southern Research Institute – BSL-2/3 containment assays for viruses and bacteria

Specialized chemistry centers: University of Kansas - HT analytical, preparative scale synthesis; virtual library enumeration; in silico properties. Vanderbilt University - HT analytical, preparative scale synthesis; DMPK; virtual library enumeration; in silico properties.

Access to local HTS labs can be obtained at: <http://www.slas.org/screeningFacilities/facilityList.cfm>. There are multiple opportunities for funding for HTS, released by the NIH in the last two years.

Dr. Parisier, Office of New Drugs, Rare Diseases Program Center for Drug Evaluation and Research (CDER) FDA, described the relatively new efforts at FDA concerning rare diseases. The rare diseases program was founded in the office of new drugs in February 2010. The program focuses on facilitating, accelerating and supporting rare disease drug development and approvals. Numerous programs and initiatives were put in place, including: Education and training programs; biomedical and regulatory science development; policy, procedure, guidance/advice development, collaboration across broad areas and stakeholders. The program is expected to expand in FY 2013, with additional RDP personnel, RD liaison in CBER, communication liaisons in CDER Office of New Drugs.

Most RD drug developers are small companies, researchers, academic institutions and/or disease advocacy groups. A newly formed NIH CC-FDA CDER Task Force will focus on : 1) Education and training for NIH intramural investigators (NII); 2) Early engagement between NII and CDER staff; 3) Identify and address systemic/recurring issues that may impede clinical research; 4) Emergency INDs.

Expanded Access Submissions Received by CDER CY 2010

New INDs	Requests Received	Allowed to Proceed	Denied
Emergency	446	434 (97)	12 (3)
Single Patient	428	428 (100)	0
Treatment	0	0	0
Intermediate Size	1	1 (100)	0
Protocols Submitted to Existing INDs			
Emergency	0	0	0
Single Patient	15	15 (100)	0
Treatment	6	6 (100)	0
Intermediate	4	4 (100)	0

Figure 7. Emergency Investigational New Drug (E-IND) history at the Office of New drugs (2010).

Session 4: Product Development Activities of the Industry – Lessons Learned

Co-Chairs: Danuta Krotoski, Ph.D. (NICHD) and Gregory Enns, M.D. (Stanford University)

Translation of basic findings to effective evidence-based interventions for patients with primary mitochondrial diseases requires a partnership between, academia, government and industry, as it is this latter sector that brings products to market. This session was organized to transmit key experiences in the development and marketing of products from a range of pharmaceutical companies. These included companies developing products for primary mitochondrial diseases (Edison Pharmaceuticals), products that may have application for treatment of primary mitochondrial diseases (Colby, Cardero Therapeutics) and treatment for related disorders (ALS) utilizing neuroprotective compounds (Knopp Pharmaceuticals). In addition, strategies and lessons learned from successfully bringing ultra orphan drugs to market (Biomarin) and an analysis of quantum evidence required for FDA approval for orphan drugs were conveyed.

The Panel addressed the special challenges for development of treatments for mitochondrial diseases. For example, are there appropriate strategies for developing protocols for small populations and heterogeneous phenotypes? How can clinical trial participants be identified? Do current clinical trial designs and FDA guidelines meeting industry's needs? What resources would be especially important for the success of intervention trials in this population? Based on both presentations and discussion the following items were viewed by industry representatives as being of particular importance in bringing treatments to market.

Identification of biomarkers and other assays for screening panels of molecules for treatment of primary mitochondrial diseases.

A barrier to development of effective treatments for mitochondrial diseases is the lack of a consensus among academic researchers as to what constitutes a reasonable set of assays by which a panel of modified molecules could be screened. In addition, there is a need for biomarkers to measure clinical endpoints or serve as surrogates for effective outcomes. This is a particularly challenging in this disease group where there is great variability in clinical and biochemical phenotypes. Conventionally in many areas of drug development, there are tools to measure a response to therapy based on the underlying disease pathophysiology so that a more effective drug can be created.

Establishment of a patient registry and central repository of human samples

Availability of and quick access to patients with a specific type of primary mitochondrial disease is a particular challenge, as individually these disorders are rare. The speakers identified the crucial need for patient registries as an important resource for linking patients with investigators and making them available to participate in clinical trials. In addition, patient registries provide the needed infrastructure to perform natural history studies (see below). The availability of such registries in other rare diseases, such as phenylketonuria greatly accelerated the conduct of clinical trials and the development of the recently approved drug, Kuvan. To this end, UMDF and NAMDC have partnered in establishing a clinical patient registry with the goal of linking patients with investigators and alerting them to opportunities for participation in upcoming studies. In addition, patient biorepositories provide access for clinical researchers to tissue samples to identify biomarkers that can be used in treatment trials and drug development. A mitochondrial disease biorepository has been established by the Mayo Clinic in 2009 and is available for translational studies. However, the costs associated with bringing in patients, data entry, and long-term maintenance of these registries and biorepositories can be high and need sustained support. Interested companies could partner with NORD to provide support for such registries.

Initiation of natural history studies for targeted therapeutics and clear outcomes

Natural history studies are particularly important in rare disorders that have heterogeneous manifestations. For small populations, where traditional large randomized clinical trials are not possible, the availability of detailed information about normal progression of disease can provide important data regarding the effectiveness of an intervention. Availability of clinical data that clearly document the natural history of a given disorder provide allows for rational design of potential endpoints in therapeutic trials. In addition, if the natural history of a disorder is known, it may be possible to employ a relatively small number of subjects in order to reach clinically significant outcomes.

Development of clinical trial designs for small populations that go beyond the randomized clinical trials.

The RCT may not always be an achievable approach for studies of rare diseases and, to that end, there is a need for small study designs. A number of successful approaches were presented, including the experience of a Biomarin study that utilized a clinical survey as a basis of a phase II study for Galsufase treatment of MPS VI (mucopolysaccharidoses VI). The survey was used to establish a clinical endpoint variable appropriate for the entire affected population and the endpoint allowed each participant to serve as his or her own control. Other examples were presented from an analysis of the quantum of evidence required for FDA approval of drugs for rare disorders by Frank Sasnowski (1). Utilization of the flexibility that FDA has built in for approval of products to treat rare disorders, the establishment of the Orphan Drug Act of 1983 and subsequent formal FDA policies and statements, including recognition of its exercise of flexibility for therapies for persons with Rare Diseases (1), were reviewed. NORD prepared an analysis cataloging the nature and scope of orphan drug precedents that illustrated the FDA's flexibility. The study demonstrated that 67% of the 135 orphan drugs approved by FDA from 1983 to 2010 resulted from some exercise of FDA flexibility in applying the statutory standard for evidence of effectiveness (Sasinowski, FJ 2012 <http://dij.sagepub.com/content/46/2/238.citation>). For serious life-threatening diseases, the FDA evaluated clinical data ranging from compassionate use to considerable benefit and also considered the magnitude of the effect. Increased knowledge about disease progression through natural history studies, for instance, could accelerate approval. However, it was emphasized that the required evidence for each treatment was evaluated on a case-by-case basis.

Partnerships to support and sustain clinical trials for rare conditions

A clear need is to identify strategies for partnering with industry to fund clinical trials for rare disorders. Products developed for rare diseases initially may have small markets, and thus may be of limited interest. However, there would be greater interest if products had broader application. This may be the case for mitochondrial diseases, because mitochondrial dysfunction may be a component in relatively common chronic diseases, as well as the aging process. The potentially accelerated approval for rare diseases, however, raises a conundrum since the period for exclusivity for "blockbuster" drugs begins with approval of usage for the rare disorder. Ultimately, support/funding for trials and selection of products depend on the potential market, but funding, especially by advocacy groups, can drive company choices.

Session 5: Opportunities and the Need for Research Collaborations and Information Sharing Among Research Partners

Co-Chairs: Petra Kaufmann, M.D., M.Sc. (NINDS) and Michio Hirano, M.D. (Columbia University)

Dr. Petra Kaufmann, Director of the Office of Clinical Research at the National Institutes of Neurological Disorders and Stroke (NINDS) identified significant challenges in collecting clinical data and outcomes that include: lack of data standards, inconsistent implementation of NIH data sharing policy, and inefficient use of single-study data collection instruments. To improve efficiency and cost-effectiveness of future clinical studies, NINDS has initiated efforts to standardize data collection through the use of common data elements (CDEs), which can be adapted for specific studies through disease-specific CDEs. The NIH ORDR is collaborating with NINDS on CDEs. In addition, valid, reliable, and easy to use outcome measures for neurological diseases need to be standardized and publically available. Potential measures include the Neuro-quality of life (Neuro-QOL, www.neuroqol.org) and the Patient-Reported Outcomes Measurement Information System (PROMIS).

Dr. Andrea Gropman, Associate Professor of Neurology and Pediatrics at Children's National Medical Center and Consultant to the NHGRI intramural Undiagnosed Disease Program, reviewed the NIH Bench to Bedside (B2B) program, which was established in 1999 to translate basic science findings into therapeutic interventions for patients. A B2B award provides up to \$135,000 annually for up to 2 years to extramural principal investigators with an existing NIH grant to collaborate with an intramural NIH partner. On behalf of the lead extramural investigator, the intramural collaborator submits a letter of intent and, if approved, is followed by a full proposal.

Dr. Michio Hirano, Professor of Neurology, Columbia University Medical Center described the North American Mitochondrial Disease Consortium (NAMDC) that is funded by a NINDS and NICHD sponsored U54 grant and the United Mitochondrial Disease Foundation (UMDF). NAMDC is one of 19 consortia in the Rare Disease Clinical Research Network (RDCRN), which is under the purview of the Office of Rare Diseases and financially supported by multiple institutes of the NIH. NAMDC is comprised of 14 sites of mitochondrial disease clinical excellence across the US and Canada. The mission of NAMDC is to facilitate mitochondrial disease research and education in partnership with the United Mitochondrial Disease Foundation (UMDF) and the NIH. Specific goals for NAMDC include development of: a mitochondrial disease clinical registry of patients; NAMDC mitochondrial disease diagnostic criteria that will be applied to all registry patients; a mitochondrial disease biobank of cells, tissues, and body fluids from patients; a pilot project program; natural history studies; and a clinical trial. NAMDC will provide critical infrastructure that is expected to facilitate mitochondrial disease clinical research and will hopefully serve as the conduit for multiple natural history studies and clinical trials.

Dr. Russell P. Saneto, Associate Professor of Neurology and Pediatrics at Seattle Children's Hospital and University of Washington, provided a summary of the NIH Centers for Translational Sciences (CTSA) program, which is under the umbrella of the recently established National Center for Advancing Translational Science (NCATS). NCATS was created to enhance the study of potential treatments for rare diseases and fast track potential drugs through the clinical trials pathway. CTSA provide many clinical services at subsidized costs for patient-oriented research. Dr. Saneto noted that the NIH realignments have led to cuts in Seattle Children's CTSA budget, which have made many approved studies difficult to fund completely. This has created a hierarchy of funded grants at this institution, big budgeted grants that are billed by the CTSA to not only cover expenses of the study but help fund smaller studies that are proposed by less endowed smaller pharmaceutical companies thereby necessitating a "Robin Hood" scenario of using larger well funded studies to help pay for smaller

studies. The mitochondrial patient community at Seattle Children's has helped raise funds to provide some of the infrastructure to help offset this decrease in budgeting. However, use of money from patient families to fund research creates ethical dilemmas, such as asking families to donate money to support small industry studies for their affected child.

Dr. Carlos Moraes, Professor of Neurology and Cell Biology at the University of Miami School of Medicine noted that numerous animal models have been developed to study defects in OxPhos, with yeast, worms, flies, zebra fish and mice. Mice are arguably the best model to study how OxPhos defects affect humans and in the last few years many models have been created. These include conditional knockouts of genes coding for critical factors necessary for the assembly of OxPhos complexes. Knockout of genes encoding factors that maintain mtDNA have also helped to better understand a number of mitochondrial diseases. Transgenic mice expressing genes that create dominant traits that can damage mtDNA have shed light on the mtDNA defects. Mice with different non-pathogenic mtDNA haplotypes (heteroplasmy), have also allowed studies to predictably alter heteroplasmy levels. Unfortunately the availability of mice with mtDNA variations is very limited at the moment, as the procedures used to create such mouse models are inefficient. Therefore, despite the relatively large number of mouse models created, there is still a need to develop models with mtDNA defects, an effort limited by the lack of methodology to engineer mtDNA modifications in vivo.

Dr. Stephen Groft, Director of the Office of Rare Diseases (ORD) at the NIH, described the International Rare Disease Research Consortium (IRDiRC), a new international co-operative effort to stimulate, better co-ordinate, and maximize output of rare disease efforts. The goals of IRDiRC are to develop 200 new therapies and means to diagnose most rare diseases by the year 2020. More than 20 governmental agencies, including NCATS and several NIH institutes, as well as private foundations have committed funds to IRDiRC, which has established a governance structure. On a national level, ORD has sponsored numerous programs to promote research and education on rare diseases. Those initiatives include the B2B program, over 1200 scientific conferences, a middle school curriculum on rare diseases, a natural history studies workshop, and new web-based Global Rare Disease Patient Registry and Data Repository (GRDR) and searchable registry of biospecimen repositories.

In summary, this session identified tenacious obstacles and exciting opportunities for translational research in primary mitochondrial diseases. Over the last two decades, we have witnessed tremendous progress in our understanding of the molecular genetic basis of mitochondrial diseases and their pathomechanisms through investigation of animal models; however, further development of these models, particularly more mouse models of mtDNA mutations will be critical to pre-clinical studies and development of therapies. Clinical studies including natural history studies and clinical trials will become more efficient through adoption of standardized common data elements and outcome measures. NCATS and ORDR will be major forces in facilitating rare disease research through national and international efforts including the B2B program, CTSAs, RDCRN, and IRDiRC. NAMDC is a particularly important program that provides registry and biorespository infrastructure for patient-oriented mitochondrial disease research. The UMDP is also engaging the international community by representing the US as a member of the International Mito Patients (IMP).

Session 6: Barriers and Opportunities to the Development and Use of New and Emerging Basic Research and Clinical Technologies

Co-Chairs: Steven Zullo, Ph.D. (NIBIB), Maren Laughlin, Ph.D. (NIDDK) and Carmen Mannella, Ph.D. (Wadsworth Center)

The talks focused on advances in cell and molecular engineering techniques for delivering macromolecules (RNAs) into mitochondria and organelles into cells, and on new imaging modalities, from intracellular to clinical. These complemented talks in other sessions that described how technological advances such as next-gen sequencing, proteomics, and high throughput drug screening are being applied to primary mitochondrial diseases.

Dr. Teitell (UCLA) described novel techniques to (1) deliver functional mitochondria into cells reproducibly and efficiently, involving a laser activated, micropipette “nanoblade”; and (2) deliver RNAs into mitochondria by fusion with a short targeting RNA sequence that interacts with mitochondrial PNPase. Imported mRNAs are able to correct respiratory defects in cell lines with defective mtDNA, suggesting considerable therapeutic potential.

Dr. Timothy Brown (Janelia Farms, HHMI) discussed how super-resolution light microscopy of cells is pushing resolution to the 20-nm range. Used alone or correlated with 3D electron microscopy, this technology is providing researchers with the ability to visualize and dissect complex biological processes and disease-related dysfunction inside mitochondria at molecular-level detail.

Dr. Craig Malloy (UT Southwestern & VA North Texas Health Care System) showed that NMR spectroscopy is a powerful nondestructive method for monitoring biochemical pathways in vivo, but suffers from very low sensitivity. Hyperpolarization of ¹³C-labeled intermediates increases their visibility by 10,000-fold. If methodological obstacles are overcome, this technology will allow direct non-invasive measurement of mitochondrial function in the tissues of patients.

Dr. Igal Madar (Johns Hopkins Medical Institutions) showed that similarly, new cationic PET agents are being developed, like ¹⁸F-fluorobenzyl triphenylphosphonium, that yield quantitative information about mitochondrial membrane potential in tissues of living people. This technology can provide important functional information for pharmacokinetic studies and help phenotype mitochondrial and other metabolic diseases in patients.

Considerable enthusiasm and excitement were generated by the technologies presented. A general consensus was expressed that more focus is needed on development of new methods for enhancing phenotyping and therapeutic capabilities.

Technology development is a complex, team-based activity, requiring interactions among biologists, engineers, computer scientists and other specialists with cultural and language differences. It occurs best in environments that value and foster it. This is the case at large NIH P41-supported centers, such as the one where the NMR hyperpolarization work is being done, and at institutions like Janelia Farm/HHMI. Technology development is generally more difficult at institutions with barriers to interdisciplinary team-science, and in labs primarily supported by hypothesis-driven R01-type grants.

Funding technology development is problematic, especially early phase activity. This has been remediated somewhat in recent years by special grant programs at NIH (within NIGMS and NIBIB) and NSF (IDBR program) that target support of promising novel technologies for biological research.

Existing SBIR and STTR funding mechanisms can help turn concepts and prototypes into user-friendly, marketable devices. Pay-lines for funding of these collaborations between academic and industry partners are considerably better than for R01 and R21 grants.

The concept was raised of establishing inter-institutional translational centers for primary mitochondrial diseases, in which certain labs could be funded as “national cores or resources” for specialized technology development and application. NIH program staff suggested that funding mechanisms for such centers already exist within certain ICs, and those PIs studying mitochondrial disease could organize and compete for them.

Session 7: General Discussion and Recommendations

Chairs: Carlos T. Moraes (University of Miami) and Vernon Anderson (NIGMS).

This section summarizes a general discussion among participants of the workshop. We list below the major barriers for the treatment of Primary Mitochondrial Diseases identified. We also make recommendations on how to overcome these.

Pre-Clinical

Better model systems to study primary mitochondrial diseases are necessary. Although the generation of mutations or ablations of nuclear genes is now a well-established procedure, the modification of the mitochondrial genome in mammalian cells continues to elude the scientific community. Therefore, better methods to manipulate mtDNA should be considered a major roadblock in the field.

Lack of understanding of mitochondrial physiology. Although some aspects of mitochondrial function are well understood, others are not. There are still a large number of factors that are either unknown or have unknown function. Mitochondrial components and function varies between tissues, and these differences are poorly understood. Likewise, the mechanisms of nuclear-mitochondrial DNA interactions are just starting to emerge. These are important barriers, which are being addressed by basic science efforts now funded by NIH. Because all future development of treatments will likely stem from this basic understanding, it is important to strengthen this effort.

The development of technological tools to examine mitochondria at the molecular level is also important to achieve the ultimate goal of understanding molecular pathways in health and disease.

Developing better drugs. Unfortunately, the drugs that have reached clinical trials have only minor effects on the development of the disease and/or significant undesirable side effects.

Monitoring response to therapeutics. Assessing the effect of drugs in patients with mitochondrial diseases remains a relatively subjective process. It would be important to develop more objective means to score improvements in phenotypes.

Testing drugs in animal models. We now have a number of worm, fly, fish and mouse models of different forms of mitochondrial diseases. Although there are limitations in testing novel drugs in animal models, much can be learned from the biochemical and physiological responses to drugs by testing them in genetically homogeneous groups of animal models. This mechanism has been underutilized by pharmaceutical companies, which view it as limited in its ability to predict success in humans.

Clinical

Registry participation is one of the most critical elements to allow potential therapeutics to be tested. The importance of supporting well-structured registries cannot be overstated. Moreover, registries allow for constant communication with patients and families, through which much is learned about the diseases. The transient funding mechanism for registries such as NAMDC is a major concern to the advancement of clinical research on mitochondrial diseases.

There is a paucity of Natural History studies in mitochondrial diseases. Because of the heterogeneity of the patient population it would be critical to engage in international collaborations and pooling patients.

Define measurable end points. Clinical heterogeneity also makes the development of objective end points challenging. Optimally, quantifiable/non-invasive end points would facilitate the enrollment of multiple centers for clinical trial studies. This would include the development of better biomarkers, for OxPhos defects.

Economic disincentives. Even though mitochondrial abnormalities are common in clinical conditions, specific mitochondrial diseases are relatively rare. This reduces the enthusiasm of pharmaceutical companies to invest substantial resources to develop and test drugs to treat mitochondrial diseases. It is important to emphasize that drugs that are effective for treating primary mitochondrial diseases may be also useful for more common degenerative diseases, which would have the potential to generate large profits.

Mechanisms to accelerate discovery and treatment options

Systems approach (Energy). Mitochondrial diseases may be better studied not focusing on specific tissues or organs. A new paradigm could be focused on the bioenergetics of tissues and organs and how a bioenergetics problem would affect the whole organism. This approach would have the advantage of better understanding the overall phenotype of patients.

Mechanisms for collaboration/coordination. Because specific mitochondrial disease phenotypes are relatively rare, it is crucial that national and international collaborative efforts are in place to pool patients. Patient advocacy groups are key partners in this endeavor. Collaborations at the basic and translational levels would also accelerate the pace of discoveries. Frequent dissemination of core facilities' capabilities and active funding mechanisms to take advantage of specialized facilities would enhance these efforts.

HTS assay development. The development of better drugs can be accelerated by obtaining initial "hits" on high throughput screening (HTS). The critical part of this procedure is the development of simple, robust, sensitive and specific assays that can be adapted to HTS. The NIH has developed mechanisms in which investigators submit their assays for HTS if backed by strong rationale and need.

Education of physicians. Although great progress has been achieved in this area, many physicians do not have a good understanding of mitochondrial diseases, leading to lengthy diagnostic procedures. A sustained education effort across specialties would improve recognition and management of patients.

Mechanisms to sustain this effort

We suggest the formation of a working group comprised of the willing chairs and co-chairs and other volunteers from the mitochondrial community, and that it be charged with developing a system for continued interaction among NIH Institutes and with the extramural community. The working group would determine the overall goals and specific objectives to be achieved through the continued interaction and identify the mechanisms that would be required. This system will help ensure that the recommendations of the Workshop are addressed and that other opportunities derived from coordination/sharing of information are pursued.