

HUMAN DISORDERS OF COPPER METABOLISM: RECENT ADVANCES AND MAIN CHALLENGES

INTERNATIONAL WORKSHOP

General information

On April 8-9, 2013, a workshop focusing on the basic and translational aspects of human disorders of copper metabolism was held at the Johns Hopkins School of Medicine, Baltimore, MD. Thirty-seven speakers and 55 other attendees from 15 countries gathered to discuss recent developments and ongoing challenges relating to Wilson disease, Menkes disease, and newly emerging copper metabolic diseases. Twenty-six posters were presented. *A unique feature of this meeting, in contrast to other copper research events, was the specific emphasis on the translational implications of basic research in the field of copper metabolism*, including a dedicated session featuring representatives of advocacy groups of affected patients and family members whose lives have been impacted by these illnesses.

The meeting featured seven major sessions, covering: Disorders of Copper Deficiency, Mechanistic Studies of Copper Biology, New Approaches for Diagnosis and Treatment, Wilson Disease: Clinical Spectrum and Treatment, Neurologic Wilson Disease, Mechanistic Understanding of Copper Overload Using Model Systems, and the Patient Advocacy Forum.

Highlights

Workshop highlights included discussion of mechanisms of copper balance in human and model organisms focused on liver, brain and gastrointestinal tract; description of a new human copper metabolism phenotype (MEDNIK syndrome); the presence of a concentrated depot of copper in the brain sub-ventricular zone; existence of molecules communicating copper status between different organs, and the role of choroid plexus epithelia in brain copper entry. The overall importance of both small and large animal models in dissecting the pathophysiology of copper metabolism disorders and for experimental therapeutics was reinforced through numerous presentations. Likewise, exciting advances in tissue copper imaging by x-ray fluorescence microscopy for copper localization were presented. From the patient perspective, the importance of developing patient registries, enhancing physician awareness, and strengthening lines of communication among clinicians, scientists, and patients were dominant themes.

Topics identified as areas of need for future research:

- The role of mitochondria in mammalian copper homeostasis in norm and disease
- Identification and functional analysis of molecules involved in regulation of well known copper-handling molecules, as a tool to better understanding of mild and complex disease phenotypes
- Precise mechanisms of copper passage into and out of mammalian brain. Functional consequences of copper misbalance on CNS function, including mechanisms of copper-deficiency neuropathy
- The role of copper in infection and immunity, especially connection(s) between copper deficiency and hematologic changes including altered white blood cell production;
- Identification of molecules that mediate copper delivery to cellular uptake systems and removal of copper from the body as an instrument for better control of copper levels in tissues
- Metabolic and tissue specific roles of copper, including potential signaling function.
- In terms of managing clinical illness, questions and challenges remain concerning the early diagnosis of Menkes and Wilson disease, the genetic and nutritional modifiers that may influence these phenotypes, and new therapies for their challenging neurological manifestations.

Other areas of investigation that are underdeveloped in the field include biomarkers for copper deficiency and overload states. Metabolomics, screening, the influence of the microbiome, and other system biological approaches should be expanded to identify new regulators of copper metabolism and cellular responses to deficiency and excess metal. Population genetics may offer insights into improvements of testing for disorders of copper metabolism. Application of metal-specific imaging such as PET-CT, which permits evaluation of copper fluxes in living organisms, should be developed further for both diagnostics and monitoring of treatment efficacy. Additional topics include: systemic and metabolic effects, link between copper and lipid metabolism, innovative treatments including cell and gene replacement therapies, new imaging strategies for clinical use, and better techniques for monitoring copper misbalance. *An all-important objective is developing ways to build stronger bridges from basic and clinical science to patients.*

SESSION SUMMARIES

Session I. Disorders of copper deficiency

Session Chair: Stephen Kaler

Other Participants: Tracy Nevitt, Joseph Prohaska, and Carlo Dionisi-Vici

Following a keynote lecture by Dr. Dennis Thiele, the opening session focused on copper deficiency disorders, including Menkes disease and MEDNIK syndrome, a recently identified phenotype associated with aberrant function of an adaptor protein complex, AP-1, which mediates proper trafficking of the two main transmembrane copper transporters, ATP7A and ATP7B. New findings on the effect of copper deficiency on white blood cell production and immunity were presented. The global effects of mammalian copper deficiency were also examined.

Session II. Mechanistic studies of copper deficiency

Session Chair: Michael Petris

Other Participants: Ling Yi, Jonathan Gitlin, and Eric Gaier

Speakers in the session on mechanistic studies of copper deficiency discussed brain-specific and motor neuron-specific ATP7A knockouts in mice, recent progress in understanding ATP7A-related isolated distal motor neuropathy, zebrafish models of copper deficiency due to mutant copper ATPase molecules, and the role of peptidyl-amidating monooxygenase, a copper-dependent enzyme, in mammalian brain development and function. A common theme was the importance of innovative animal models for analyzing and illuminating important aspects of human copper metabolism.

Session III. New approaches for diagnosis and treatment

Session Chairs: Martina Ralle and Dominik Huster

Other Participants: Stephen Kaler, Stefan Vogt, Fanguy Peng, Bart Van de Sluis, and Jaekwon Lee

This session was focused on progress in fundamental research as a basis for novel approaches to diagnosis and treatment of human disorders due to copper misbalance. The presenters covered a wide spectrum of topics: from new discoveries in copper biology, recent advances in copper imaging in biologic samples to current success in viral-mediated gene therapy in mouse models of Menkes disease. Emerging imaging techniques such as X-ray fluorescence microscopy and PET-CT in living organisms have provided new insights into copper handling.

Translational research using yeast, mouse and canine models have identified new key players in copper biology such as COMMD1, and new links between copper and cholesterol metabolism.

Session IV. Wilson disease: clinical spectrum and treatments

Session Chair: Michael Schilsky

Participants: Aftab Ala, Sihoun Hahn, Peter Ferenci, Michael Schilsky, Sanjeev Gupta, Julian Mercer, Anil Dhawan, Pascale Delange

This session began with a review of disease pathophysiology that emphasized on the wide spectrum of clinical presentations of Wilson disease. It has been presumed that Wilson disease has near complete penetrance with respect to phenotype in individuals with homozygous or compound heterozygous ATP7B alterations. Recent data suggests a higher gene frequency of ATP7B mutations than previously appreciated; in fact, based on genotype, a prevalence as high as 1:5,000 is predicted. Since the ascertainment of Wilson disease in populations is not this high, lower penetrance is now suspected.

Molecular sequencing for ATP7B mutations has aided disease diagnosis and is now recommended as the primary screening modality for first-degree relatives of affected patients in whom ATP7B mutations have been demonstrated. There are few secure genotype and phenotype correlations given the large number of disease-specific ATP7B mutations, although in some instances specific mutations were more frequently associated with neurological disease. Other non-genetic factors may influence Wilson disease presentation. For example, gender appears to influence clinical presentation: females present with hepatic disease more often than males. More attention to factors that modify disease presentation, including other genes involved in copper transport, responses to injury and oxidative stress, and other environmental factors may yield useful information about the variable phenotypes observed in Wilson disease.

Regenerative medicine holds great promise for the future of disease treatment. It may someday supplant whole organ transplantation. Cell transplantation of adult hepatocytes reduces copper content of the liver and improves hepatic histology in animal models of Wilson disease. At present, it is still necessary to stimulate the selective growth of implanted cells relative to native liver cells to achieve adequate repopulation with a functional cell mass. Stem cells offer an alternative to adult liver cell transplant, and these may be acquired from embryonic or pluripotent cells. Future manipulation of stem cells may enhance function. Alternatively, new methods for gene transfer may permit *ex-vivo* therapy for re-implantation of hepatocytes whose function has been corrected or enhanced.

Non-invasive imaging studies utilizing radiocopper complexes containing either the amino acid histidine, which better targets copper to cells, or to specific proteins, helps more specifically target copper to hepatocytes and can help establish a diagnosis of Wilson disease. Imaging with radiocopper complexes provides a non-invasive way to demonstrate disease correction that may be useful for monitoring cell based or gene therapies for Wilson disease.

The ATP7A and ATP7B proteins are expressed in different cell types but function to transport copper at the basolateral and apical membranes respectively of the polarized cells in which they are expressed. To determine whether these proteins may complement each other's function in a mammalian model, human ATP7A was introduced into the liver of ATP7B deficient toxic milk (tx) mice by crossing the toxic mouse with transgenic mice expressing the Menkes disease gene product. The ATP7A protein expressed in the liver caused copper to leave the liver, likely

by the basolateral and not apical (canalicular membrane) and corrected the copper induced liver injury for tx mice. However whether ATP7A induced copper efflux from the liver leads to an increase in extra-hepatic manifestations of copper toxicity in these animals due to increased circulating copper or whether ATP7A can mediate hepatocellular copper incorporation into ceruloplasmin remains to be determined.

Human hepatocyte transplantation is being tested as an alternative or bridge to liver transplantation in a select group of children with metabolic disorders and acute, non-cirrhotic liver failure. The number of cells transplanted usually represents about 5% of total liver mass. The safety of the procedure has been established and the clinical results suggest short-term improvement in disease phenotype. However, cell function often declines with time from the cell transplant. Future work is needed to standardize techniques for this procedure and to see if it can be applied to cirrhotic as well as non-cirrhotic livers.

Novel compounds for the removal of excess copper in the form of Cu(I) directly from liver cells were evaluated *in vitro*. Cu(I) chelating agents were designed using a CuS₃ coordination mode, as found in the copper-binding peptide metallothionein and these agents displayed a high selectivity for Cu(I) over Zn(II). Using a glycoconjugate with the copper chelator, the complex was targeted directly to hepatocytes via specific ligand surface receptors. Testing of this compound in animal models is ongoing and, if successful, could lead to novel, liver-targeted therapy for Wilson disease in the future.

Session V. Neurologic Wilson disease

Session Chair: Peter Hedera

Participants: Wolfgang Stremmel, Peter Hedera, Wieland Hermann

The presence of copper in the brains of Wilson disease patients is the cause of CNS injury, but this copper has been hard to target directly, since water-soluble medications typically do not cross the blood-brain barrier. To attempt to directly target the CNS, triethylenetetramine was encapsulated into liposomes with a targeting vector (cationized cBSA) attached to the liposome surface permitting delivery across the blood-brain barrier into CNS tissue. Liposomes coupled to cBSA were taken up into brain capillary endothelial cells, whereas liposomes without targeting ligand had negligible uptake. Confocal fluorescence microscopy after intravenous administration of cBSA-liposomes showed uptake into the brains of rats. These data indicated the feasibility of this strategy. However, whether this treatment approach will effectively alter cerebral copper deposits requires further testing.

Deep brain stimulation has been very helpful for some patients with uncontrollable movement disorders. The direct implantation of electrodes into the brain and devices set to deliver current to these areas that are worn externally allows control of movements in some previously untreatable patients. Only a few patients with Wilson disease have undergone this procedure. Careful selection and longitudinal monitoring of these patients should help to inform us about when and how to consider this treatment option in Wilson disease patients with neurological disease.

Patients with neurological Wilson disease have been evaluated by various imaging modalities. In T1-weighted MRI, atrophy is the predominant finding, whereas in T2-weighted MRI, lesions are detected in neurological core areas, predominantly the putamen. Some patients present with minor abnormal CNS imaging findings prior to the onset of neurological symptoms. In contrast, neurologically symptomatic patients with Wilson disease invariably manifest

abnormalities in imaging studies, and these tests can be useful for following a patient's response to treatment.

Session VI. Mechanistic understanding of copper overload using model systems

Session Chair: Dennis Thiele

Other Participants: Svetlana Lutsenko, Martina Ralle, Hans Zischka, Dennis Winge, Dominik Huster and Hille Fieten

This session highlighted recent advances in our understanding of copper imbalance in disease through analyses of the metabolic and dietary variables in phenotypic diversity. This includes recognition of downregulated lipid metabolism in association with ATP7B dysfunction and liver copper accumulation. Technical advances in copper imaging, such as metal-tagged antibodies, are allowing new insights about the distribution of copper in tissues and organs. On a cellular level, the important role of mitochondria in overall copper homeostasis is also emerging. A clear message from this session was that powerful new insights about human copper metabolism are possible through study of model organisms including yeast, mouse, rat, and canine.

Session VII. Patient advocate perspective

Session Chair: Mary Graper, Wilson Disease Association

Other Participants: Jamie Eckman, The Menkes Foundation; Carol Terry, Wilson Disease Association; Pam Meadows, Wilson Disease Association; Patricia Paulin, Nurse for Wilson disease patients. Allison Delano, Menkes disease parent; Aaron Chang, Wilson disease patient

This session provided a lively forum for the insights, questions, and recommendations of patients and patient advocates. Main goals, as stated in the session overview, were to discuss future research topics of particular concern to the patient community that have not yet been adequately studied; how best to integrate patient concerns with researcher interests; how the patient advocacy groups, e.g., the Wilson Disease Association and the Menkes disease Foundation), can best support future research projects; and finally, how best to disseminate updated information on current research initiatives to the lay community. The desired outcome is an open exchange of ideas between the medical, scientific, and patient communities concerning future directions in the field. As highlighted by one panel member, this model and approach are neatly represented by the Patient-Centered Outcomes Research Institute (<http://www.pcori.org>).

Key themes presented included:

- The need for and value of patient registries for Menkes and Wilson disease to support effective research;
- The need for early detection of both Menkes and Wilson disease through newborn screening and the associated need to increase awareness in the pediatric medical community;
- The need for more effective treatments for these conditions and for the chronic disabilities that can result from them;
- The need to facilitate translation of research outcomes to clinical practice guidelines;
- Identification of approaches to reduce patient frustration, minimize misinformation, and improve communication.

There were several other specific topics raised by the Wilson disease community. Do Wilson disease carriers exhibit symptoms? Is there a connection between autism and Wilson disease? Is hyperlipidemia more common in Wilson disease patients than in the general population? Is

there a connection between diabetes and Wilson disease? Does orthotopic liver transplantation improve neurologic symptoms?

At the session conclusion, the panel expressed its hope for better cooperation amongst the various international patient advocacy groups, and that basic and clinical researchers might form valuable research partnerships through this meeting.

Workshop Summary

Session Chairs: Svetlana Lutsenko and Eve Roberts

Remarks: Jonathan Gitlin

This session briefly summarized the key topics and findings, the possibility of creating working and productive meetings between clinical and basic scientists, as well as new themes in copper research that emerged during the conference.

FUTURE DIRECTIONS FOR THE AREA OF HUMAN COPPER METABOLISM

Here we summarize the results of the discussions during and after the workshop, including post-sessions meeting and e-mail exchanges between the members of the organizing committees while preparing the current white paper.

We plan to publish main proceedings of the Workshop in a peer-reviewed journal, with the goal of generating a high quality volume (or volumes) on current knowledge of human copper metabolism. This would serve both as a record of this unique conference, and as a benchmark reference for future meetings. The *Annals of NY Academy of Sciences* is one potential venue for this goal.

Other highlights (such as identifying key areas such as copper in the CNS, imaging, models, new therapies) will be publicized via the Wilson disease and Menkes disease association websites. This would enable submission of comments and suggestions from clinicians, patients, and families on what they like to see developed further.

A developing future direction is **a formal recommendation that NIH generate a program announcement about the role of copper in the CNS as a special area of interest.** This could include essentially all the important directions discussed at this workshop. To facilitate entry of new generation of scientists, in such new funding mechanisms preference can be given, for example, to collaborative projects involving young neuroscientists who wish to work with established copper research scientists, or conversely, young copper research scientists who wish to work collaboratively with established neuroscientists. Current K01 awards do not accomplish this goal, because two areas (copper chemistry/biology are sophisticated modern neuroscience) largely do not overlap. Explicit interest from the NIH is likely to facilitate collaborative interactions, may bring new and desirable expertise to the field, and allow more rapid progress. Additional research utilizing a systems biology approach to normal and abnormal copper metabolism could also benefit the field.

We also suggest that a "Metals in Cells" NIH study section be established. Currently, the review of copper research grants is not consolidated. A committee comprised of experts from the field would allow focused review and identification of the most important and pressing translational research questions.

We recognize the growing importance of establishing firmer connections among the copper

disease clinical communities of North and South America, Europe, and Asia. This will require time, funding, and a collaborative spirit.

We should develop a mechanism by which presenters from this workshop may share research discoveries and exchange ideas, perhaps through a closed group on *Facebook* or *Linked-In*. This also would aid the workshop organizers in planning future copper research meetings.

We should consider expanded involvement between copper disease patients and the copper research community, such as a laboratory "open house," to which copper disease association patients and families are invited to visit. In addition, there may be opportunities to link the several clinical centers of excellence in Wilson disease with the basic science research community.

We recommend a follow-up Workshop on Human Disorders of Copper Metabolism in approximately within 3 to 4 years.

We need to establish an organized approach to disseminate emerging research accomplishments in the field to the copper disease patient communities. We also need to establish information conduits that enable researchers to appreciate the "real-life" issues of patients with copper-related illnesses.