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Translational research investigations on ATP7A, an important human copper ATPase

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Abstract

In the more than 40 years since copper deficiency was delineated in pediatric subjects with Menkes disease, remarkable advances in our understanding of the clinical, biochemical, and molecular aspects of the human copper transporter ATP7A have emerged. Mutations in the gene encoding this multitasking molecule are now implicated in at least two other distinctive phenotypes: occipital horn syndrome and ATP7A-related isolated distal motor neuropathy. Several other novel inherited disorders of copper metabolism have been identified in the past several years, aided by advances in human gene mapping and sequencing. In this paper, I review the history and evolution of our understanding of disorders caused by impaired ATP7A function, and outline future challenges.

Keywords

ATP7A; Menkes disease; occipital horn syndrome; viral gene therapy; human copper metabolism

Introduction and history

ATP7A is now recognized as a critical copper transport protein with multiple important cellular functions.¹ In 1962, when John Menkes reported the clinical and biochemical phenotype underlying a X-linked recessive disorder of growth retardation, neurodegeneration, and peculiar hair, both the protein and its role in mammalian copper metabolism were entirely unknown.² Cloning of the gene responsible for this condition and the allelic disorder, occipital horn syndrome (OHS), was achieved in 1993, revealing the importance of ATP7A, a highly conserved ion-motive ATPase.^{3–6}

Menkes attended medical school at Johns Hopkins Medical School, returned as faculty, and ultimately became head of Pediatric Neurology there. It was at Johns Hopkins that Menkes first crossed paths with David Danks, the Australian physician who had come to work “under the dome” with medical geneticist Victor McKusick for 9 months, from October 1961 to June 1962 (Fig. 1). Ten years later (1972), Danks established the initial connection

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between Menkes disease and copper deficiency, based in part on his keen memory of affected patients seen during that sabbatical.⁷ Thus, the decision to host the April 8–9, 2013 conference “Human Disorders of Copper Metabolism: Recent Advances and Main Challenges” at the Johns Hopkins School of Medicine, arranged intrepidly by Svetlana Lutsenko of the Johns Hopkins Department of Physiology, was most appropriate. Among the exceptional group of colleagues assembled for the meeting, the presence of three winners of the David Danks Award for research in copper metabolism (Mercer, Winge, and Thiele) further extended the connection between the gathering and the field’s original leaders.

In keeping with the intention of that conference to identify knowledge gaps and expose new translational research directions, this article will review what we are learning about human copper metabolism from both patients and laboratory experiments. In addition, areas that might be fruitfully explored further in the future will be highlighted throughout the review.

Clinical phenotypes associated with disturbed copper metabolism

ATP7A is a multifunctional molecule required for both copper transport within cells and copper exodus from cells (Fig. 2). Mutations in *ATP7A* can cause any of three distinctive phenotypes, depending on the molecular defect.⁸ Menkes disease presents in infants between six weeks and one year of age with hypotonia, seizures, developmental delay, brain atrophy, and coarse, lightly pigmented hair that rubs off easily.^{2,8–10} Jowly facies, lax skin and joints, decreased bone density, bladder diverticula, gastric polyps, venous aneurysms, cardiac defects, vascular tortuosity, and blue irides are additional common clinical findings.^{11–16} Low serum copper and ceruloplasmin, abnormal plasma and CSF neurochemicals, and increased urine β -2-microglobulin are typical biochemical findings.^{17–20} Prognosis for patients affected with this illness is difficult without very early diagnosis and institution of copper replacement treatment.²⁰ Future research (and public health) goals for this condition include newborn screening for early detection and brain-directed viral gene therapy to provide a normal, functioning version of ATP7A to the brain.²¹ Adeno-associated viral vectors have emerged as safe and superb vehicles for gene transfer.²² The brain, as an immune-privileged target, is favorably protected from the effect of neutralizing antibodies against the AAV capsid. These factors, coupled with the inadequacy of current treatment, should render Menkes disease a bona fide candidate for this treatment approach in human subjects within the next several years.²³

OHS is a milder allelic variant of classic Menkes disease.^{6,24} Age of onset is between 3 and 10 years, and physical findings include coarse hair and lax skin and joints. X-ray radiographs reveal occipital bone exostoses and hammer-shaped clavicular heads. Other findings may also be present, including bladder diverticula and vascular tortuosity, as well as symptoms of dysautonomia such as fainting spells, dizziness, orthostatic hypotension, abnormal sinoatrial conduction, nocturnal bradycardia, and bowel or bladder dysfunction, which are all related to reduced activity of the copper-dependent enzyme dopamine β -hydroxylase (DBH). The connective tissue manifestations (skin and joint laxity, vascular tortuosity, bladder diverticula) are ascribed to deficiency of lysyl oxidase, another copper-dependent enzyme. Like DBH, lysyl oxidase is processed through the intracellular secretory

pathway, through which copper is normally incorporated into nascent apoenzymes. Biochemical features of OHS include abnormal plasma and CSF neurochemicals and low-normal serum copper and ceruloplasmin levels. The less severe neurodevelopmental component of OHS compared to classic Menkes disease reflects the presumed capacity for considerable ATP7A-mediated copper transport in the brain, due to less disabling *ATP7A* mutations. These include “leaky” splice junction defects⁶ and hypofunctional missense mutations.²⁴ Treatment is not usually offered for this variant, given the overall mild neurological effects; however, the norepinephrine pro-drug L-dihydroxyphenylserine (L-DOPS) could potentially be highly effective for the dysautonomia suffered by these patients.²⁵

ATP7A-related distal motor neuropathy²⁶ is distinctively different from both Menkes disease and OHS. This phenotype is highly reminiscent of Charcot–Marie–Tooth hereditary neuropathy type 2, yet is caused by unique missense mutations in *ATP7A*.²⁷ Findings in the first two reported families suggest that the age of onset may range from 5 to 50 years. Slowly progressive weakness and atrophy of distal muscles are the neuromuscular hallmarks, and affected patients manifest gait abnormalities, foot drops, and abnormal nerve conduction studies. In contrast to Menkes disease and OHS, there are no specific biochemical abnormalities present. Therapeutic strategies for this condition await a more precise understanding of the underlying pathophysiological mechanisms, which are currently under investigation.²⁸

Other diseases affecting the activity and function of ATP7A

Recently, two autosomal recessive conditions have been recognized which involve gene products that may indirectly affect the function of ATP7A. Huppke–Brendel syndrome is caused by mutations in an acetyl CoA transporter, *SLC33A1*, needed for acetylation of one or more copper proteins.²⁹ MEDNIK syndrome is caused by mutations in the $\alpha 1$ subunit of adaptor protein complex 1 (AP-1), which leads to detrimental effects on ATP7A trafficking.³⁰

Summary and conclusions

The past two decades have witnessed a remarkable growth in our knowledge and understanding of eukaryotic copper metabolism. Appreciation of the basic pathways that guide cellular copper uptake, transport, and export has reached a reasonable level; however, we know considerably less about the precise mechanisms that underlie the neurological consequences of disturbed copper homeostasis and the ideal remedies to treat these consequences.

Issues that appear ripe for further research include the role of ATP7A at the blood–brain and the blood–CSF barriers and the specific functions of this transporter in glutamatergic, acetylcholinergic, and other neurons. The roles of ATP7A in axonal and synaptic physiology remain obscure, as does the question of whether the ATP7A-related distal motor neuropathy involves copper deficiency or excess. Among other translational research questions to be addressed are whether population-based newborn-screening assays can be developed to test

for *ATP7A* disorders, and whether gene replacement approaches in mouse models are applicable to human patients with large deletions or other severe loss-of-function *ATP7A* mutations. Answers to these ongoing research questions will have important implications for patients with *ATP7A*-related illnesses and their families.

Translational research investigation of *ATP7A* intersects the fields of biochemistry, cell biology, genetics and gene regulation, neuroscience, and structural biology. While many uncertainties remain, the pace of discovery concerning this fascinating molecule across multiple disciplines has quickened noticeably in recent years, auguring well for substantial future advances. The history of investigation into human copper metabolism includes crucial clinical observations by John Menkes and David Danks. As with the intentions of that remarkable pair, our current collective research efforts point toward an even greater understanding of *ATP7A*-related copper transport diseases that will translate to improved human health.

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Figure 1.

The original ATP7A translational research team. The medical careers of Drs. John Menkes and David Danks overlapped in the 1960s under the dome of Johns Hopkins Hospital (top).

Figure 2.

ATP7A is a mobile and versatile copper-transporting ATPase. (A) Confocal images of HEK293T cells transfected with a Venus-tagged version of wild-type *ATP7A* relocalizes from the *trans*-Golgi network under basal copper cellular conditions (0.5 μ M) and to the plasma membrane under increased copper (200 μ M). (B) Total internal reflection fluorescence (TIRF) microscopy in normal human fibroblasts stained with an antibody against ATP7A illustrates the same phenomenon. TIRF selectively illuminates the plasma membranes of cells.