

Comprehensive insights into Menkes disease: Genetics, pathophysiology, animal models, diagnosis, and therapeutic strategies

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Abstract

Menkes disease (MD) is an inherited disorder affecting primarily the males which is X-linked recessive in inheritance pattern due to mutation in ATP7A gene that codes for copper transporter protein. This results in neurological complications and connective tissue lesions that make affected males succumb to death. MD can be classified as the classical and Occipital Horn Syndrome (OHS) varieties, though the severity and early presentation of the classical MD is more pronounced. Diagnostic assays include determination of serum copper and ceruloplasmin concentrations; neurochemical studies are also performed. The standard treatment usually entails the use of copper supplements with copper – Histidine being one of the most effective. Therefore, it is necessary to investigate the molecular pathophysiology of ATP7A disorder to come up with different therapies. Animal models such as zebrafish, fruit flies etc. are beneficial to know about MD pathology to get treatment. In summary, the early and accurate diagnosis of MD, followed by immediate treatment, plays a critical role in successful management of the disorder.

Keywords: Menkes disease; ATP7A Gene; Copper metabolism; Occipital Horn Syndrome (OHS); Copper-histidine therapy

1. Introduction

Menkes disease (MD) is an X-linked recessive demyelinating disorder leading to progressive mental retardation, seizures, and early death in affected males because of mutations in the ATP7A gene, a copper-transporting ATPase. This enzyme is also important in the ability to identify where copper is required in the body and then deliver it there. The overall incidence ratio according to Vagero et al. ranges from 1/100000 to 1/250000 live births. This results in low levels of copper in the cell, which affects the movement of copper from enterocytes, and the activities of copper-containing enzymes. It arises in several forms, which include the nonsense mutation, missense mutation, insertion, and deletion. There is a similar disease named Wilson disease, which is caused due to the mutation in ATP7B gene that encodes a similar protein to that encoded by ATP7A. Due to these findings, evaluation of the molecular mechanisms underlying ATP7A and ATP7B could help elucidate the pathology of these diseases (1). Copper dyshomeostasis in Menkes disease involves copper deposition within intestinal enterocytes and decreased levels of circulating, hepatic and brain copper. This deficiency interferes with cuproenzyme functions, leading to neuronal damage, arterial lesions, hair and connective tissues pathologies. The inability of parenteral copper to offer effective neurological treatment is due to the deficiency of copper in the brain after the blood-brain barrier has formed. The disorder disrupts the balance of copper in neurons and glial cells, thus affecting the transportation of copper to organelles and also lowering the functions of cuproenzymes. In terms of treatment, the goal is to transport copper into intracellular compartments: with promising therapies to use lipid soluble chelators to transport copper through the cell membranes (2). Menkes disease is a group of X-linked genetic disorders characterized by impaired and deficient copper transport which leads to worsening of neurological conditions and death. It is actually a very limited disorder, affecting about 2 in 100, 000 male

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births. This makes genetic analysis very challenging since usually-affected females are heterozygous and thus asymptomatic most of the time. Carrier detection is done in combination with copper uptake in formed cells, but this process is not perfect. Despite its complex clinical manifestations, recent genetic studies propose that Menkes disease is inherited in an autosomal recessive manner, and approximately 30% of the affected males may be the result of spontaneous mutations. To sum up, there is no indication of an increase in the paternal age at mutation through the studied lineage. The disease is predominant in male because affected females are almost negligible due to its genetic death formula (3). Along these manifestations significant learning disability, hypotonia at birth, intractable epilepsy, and neurological worsening are seen. Children develop typical hair temporalization including depigmented and brittle hair as well as they have certain facial features like pale skin and chubby cheeks. People with hansen also display skin that is loose and joints that are also loose, they get osteoporosis and frequently break out into Bone fractures too. There are combined features of arterial disease, hypothermia, multiple diarrhea, bladder diverticula, and UTI. This diagnosis is likely given the patient's very low level of serum copper and ceruloplasmin. Parenteral copper treatment may be of benefit in some ways when administered early on, however, neurological abnormalities which are typical for this disease remain unchanged if parenteral copper treatment is not given at the early stages of the disease. There are different forms of the disease, and its mild form and internal form or, as it is also called, Occipital Horn Syndrome (OHS) are distinguished (4). The accuracy of neonatal diagnosis of Menkes Disease: A new diagnosis pathway is the plasma catecholamine analysis, the ratio of dopamine/norepinephrine is $> 0.2/C$ and dihydroxyphenylacetic acid/dihydroxyphenylglycol ratios > 5 ; demonstrated 100% sensitivity and specificity in studies. Copper-histidine therapy initiated at an early age is known to have favourable effects on survival and neurodevelopment, with a number of patients presenting normal results. Genotyping of the ATP7A gene is only advised when catecholamine analysis is not feasible. Although prenatal diagnosis does not have the same level of evidence and certainty, it can provide the opportunity for early treatment or prevention, or terminate the pregnancy. In conclusion it can be concluded that early and accurate diagnosis as well as initiating antiviral therapy as soon as possible are imperative for dealing with MD(5).

2. Cellular and whole-body copper metabolism -

After iron and zinc copper is the third most abundant trace element in the body and involved in several metabolic process such as cellular respiration (cytochrome-c oxidase), maturation of peptide hormones (peptidyl alpha amidating enzyme), free radical scavenging (superoxide dismutase), neurotransmitter biosynthesis, cross linking of elastin, collagen, and keratin. Copper can exist in two oxidation states Cu(1) and Cu(2). Copper uptake across the plasma membrane via CTR1, an energy independent membrane transporter. In cytoplasm copper is bound to small proteins such as glutathione, metallothionein or copper-specific chaperones such as CCS, ATOX1 and COX17. CCS targets copper to superoxide dismutase, residing in the cytosol or in mitochondria. ATOX1 guides copper to trans-Golgi network (TGN) and it is incorporated into copper-requiring enzymes synthesised in the secretory pathway. COX17 guides copper to mitochondria and is incorporated into COX. These copper chaperones bind with copper and protect cells from toxic effects of the free ions. In the TGN copper specific two homologous membrane bound ATPase are present such as ATP7a and ATP7B that responsible for ATP driven efflux of copper from cell and transfer copper across the membrane into the lumen of TGN, where it is delivered to secreted enzymes. Under normal physiological concentration ATP7A is localised to TGN, if concentration is increased ATP7A is translocated to the vesicles or to the plasma membrane. Almost in every organ ATP7A is expressed except the liver where ATP7B is predominantly expressed. The copper transporters CTR1, ATP7A, ATP7B are all expressed in brain barrier fractions and involved in transportation of copper in the brain. In MD patients' copper is trapped in both blood-brain barrier and blood-cerebrospinal fluid barrier, therefore the neurons and glial cells are deprived of copper, leading to low copper level in the brain(6). Clinically, MD is Cu deficiency syndrome which is caused by the dysfunction of multiple Cu-dependent enzymes and also shows low levels of serum Cu and ceruloplasmin, which is the major copper-containing protein component in serum(7). Under normal dietary conditions, the gastrointestinal (GI) tract in humans absorbs ~30%–40% of ingested copper. Copper is present in various GI fluids such as salivary, gastric, pancreatic, duodenal, and bile. The copper is first absorbed from the distal small intestine and reabsorbed into the circulation in its protein-bound form and reaches to the liver and kidney. Once copper enters the body, it is transported to different organs bound to plasma protein carriers such as ceruloplasmin, transcuprein, and albumin. Next copper is transported from the liver (and perhaps kidney) to other organs, including the brain, heart, and skeletal tissue. A little amount of copper is secreted into the bile and is lost through the faeces. Most of the copper reaches the liver and kidney is backed to plasma bound to ceruloplasmin. Low levels of brain copper in MD affect catecholamine pathways and lead to abnormal catecholamine metabolite levels in blood and body fluids. The ratios of the dopamine metabolites, dihydroxyphenylacetic acid (DOPAC) and the catecholamine precursor dihydroxyphenylalanine, to neuronal metabolite of norepinephrine, dihydroxyphenylglycol (DHPG), were increased above control values and indicating the partial dopamine β -hydroxylase deficiency.(8)

3. Structure and importance of ATP7A

ATP7A is a P-type ATPase that is energy utilising membrane proteins functioning as cation pumps. Also includes Na⁺/K⁺ and H⁺/K⁺ pumps as well as plasma membrane and sarcoplasmic reticulum Ca²⁺ pumps. ATP7A transport copper across a membrane and use energy released by ATP hydrolysis and involve domain specific for binding. These domains are nucleotide binding domain(N-domain), phosphorylation domain(P-domain) and activation domain (A-domain). For translocation and transport different motifs are required for recognition, binding, and translocation of the metal across a membrane. These motifs contain cysteine residue residues, and play an important role in copper binding(9). At the N-terminus ATP7A has six metal-binding domains (MBD1-6) each containing a consensus MTXCXXC motif. Copper binds to these domains in Cu(1) form. The first four metal binding domains have regulatory functions. Through this domain the interaction between ATP7A and the copper chaperone ATOX1 occurs. The n-domain and P-domain reside between TMD6 and TMD7. N-domain binds ATP and the gamma phosphate of ATP and then transferred to invariant aspartate residue(D) in the DKTG motif, which resides in P-domain. After translocation of copper through the membrane, P-domain is dephosphorylated. A-domain is present between TMD4 and TMD5, including invariant TGE motif, where the glutamate residue (E) mediates dephosphorylation of the phosphorylated intermediate. At the C-terminal ATP7A domains contain conserved di-leucine residue (LL), which is important in the retrieval of the protein from the membranes. ATP7A is anchored through 8 hydrophobic transmembrane domains. The CPC motif within TMD6 plays an important role in the transfer of copper(10). Copper transporting ATPase ATP7A is essential for mammalian copper homeostasis. Due to the loss of ATPase activity, it leads to the fatal Menkes disease and various other pathologies. In cells, inactivation of ATP7A disrupts copper transport from the cytosol into the secretory pathway. ATP7A activity protects mitochondria from excessive copper entry and maintains the mitochondrial redox balance(11). Menkes disease is a lethal neurodegenerative disorder caused due to the absence or dysfunction of a putative copper transporting ATP7A (and ATP7B), member of a large family of p-type ATPase encoded on the X-chromosome. Polyclonal antisera were developed against a bacterial fusion protein encoding the 4th to 6th copper-binding domains of amino terminus Menkes protein in humans to elucidate the biosynthesis and subcellular localization of these proteins. RNA blot analysis and immunoblotting also used to reveal the Menkes gene expression in several cell lines. Immunofluorescence studies also help to localise this protein to the trans golgi network and a vesicular compartment (12).

4. Types of Menkes Disease

The two main type of Menkes disease includes -

4.1. Classical Menkes Disease

It is a rare X-linked recessive disorder reported to affect boys mostly, and some of the symptoms of the classical Menkes disease include neurodegeneration, developmental regression, seizures and connective tissue disorders. Common clinical manifestations include short stature with characteristic kinky hair, hypotonia, poor growth, and feeding difficulties which become apparent at 2-3 months of age. Definitive diagnosis is made by lowering serum copper and ceruloplasmin levels, and footprint of hair like pili. Its cause is a genetic defect of a protein that works on copper absorption, or more specifically, a defect of a copper-transporting P-type ATPase gene. This impacts numerous copper-associated enzymes, which, in general, enters clinical manifestation and is death caused by 3 years(13).

4.2. Occipital Horn Syndrome (OHS)

Occipital horn syndrome (OHS) is one of the genetic disorders that has been found to involve the X chromosome; the ATP7A protein. These mutations affect the synthesis and organization of proteins and lead to the abnormally structured connective tissue exhibited by the disorder, which comprises loosely hanging skin, joint hypermobility, and occipital horns that protrude from the base of the skull. Although OHS is associated with some similarities to classical Menkes disease, which is another type of disease resulting from mutations in the ATP7A gene, patients receiving this designation all in all, usually have milder symptoms. Diagnosis of OHS is usually made through clinical assessment by clinician with the presence of characteristic somatotype and can be further confirmed with genetic analysis. It mainly focuses on the alleviation of symptoms and the dealing with complications that may ensue, for instance hernias that may call for a surgical treatment. Furthermore, symptoms or coexisting disorders such as dysautonomia will often require treatment in an effort to enhance the quality of life in those who have been diagnosed with this rare disorder (14).

5. Genetic Aspect of Menkes Disease

An understanding of the genetics of Menkes Disease relates to the deficiency of the product of the ATP7A gene, initially named the MNK gene. ATP7A is a gene found to map on the X chromosome and produces a protein that transports copper and regulates its accumulation in the body. It is a disorder that occurs due to X-linked recessive inheritance that means the gene responsible for Menkes Disease is located on the X chromosome. A study on the genetics of Menkes Disease has established that the defective gene ATM contains a wide range of variation. These mutations can represent cytogenetic changes such as translocations or deletions of chromosomes, or represent effectively single sequence alterations. In a minority of cases, cytogenetic imbalances may be evident and present with balanced X; autosomal translocations or with gross aberrations in the X chromosome. In fact, point mutations are more frequent and there are numerous different variations within the ATP7A domain. Most of them cause alteration of ATP7A protein that is crucial in the uptake of copper and the subsequent transport of the metal across cell membranes; therefore, the deficiency translates to the clinical manifestation of Menkes Disease. Notably, the pattern of mutagenesis may cluster in specific regions of ATP7A gene, its exons 7-10. Defects in the exon 8 have been frequently mentioned to account for a considerable number of severe Menkes Disease cases; this emphasizes the probable role of this segment in the activity or integrity of ATP7A protein. Furthermore, Menkes Disease affects females who are regarded as having two X chromosomes but in very uncommon cases. Patients with MD can be women: X-chromosome inactivation or an Abnormal X chromosome can cause MD (1) – balanced translocations in the X chromosome in the region of the ATP7A gene (15).

6. Animal Models of Menkes Disease

6.1. Mouse Model

The mouse model of Menkes disease is the mottled mutants which results from disturbances in the murine equivalent of the Menkes gene. Their molecular analysis reports different phenotypes based on the concentration of residual MNK (Menkes protein) in the mutant males. The observed phenotypic variation is due to variability in organ-specific threshold levels of copper needed during the developmental stages. For example, extreme mutations result in fetal lethality by impaired cross-linking of connective tissue as well as vascular wrecking in Mo9H mutants. In contrast, the more moderate mutations result in the capacity of the embryo to survive through gestation but die soon after being born or their connective tissues fail throughout their lifetime. These outcomes reflect various symptoms of human Menkes disease, though fetal death is hardly widespread because people can occur without MNK during their growth. This mouse model contributes toward the understanding of Menkes disease and can highlight the importance of copper in prenatal and postnatal development(16).

6.2. *Danio rerio* and *Drosophila melanogaster* as Model of Menkes Disease

Tropical fish such as zebrafishes and fruit flies are considered appropriate non mammalian models for menkes disease. Zebrafish are best suited to study early copper metabolism with reference to human beings since they develop rather fast. Lcc mice carrying *Atp7a* hypomorphic alleles exhibit Menkes-like phenotypes; however, they still show nearly normal activities when supplied with even low levels of *Atp7a* function. Likewise, the fruit flies with mutations in *DmATP7* gene or where the ATP7 gene is knocked out for the study show lethargy and growth defects that resemble those of Menkes disease. These results indicate that nervous system tissue is involved in the conditional manipulation of *DmATP7* RNAi in the gut and an increase of lethality. These models give information about the status of Pathophysiology of Menkes disease and may be helpful in the therapeutic approach (17).

7. Diagnosis and treatment

Menkes is a lethal neurodegenerative disorder caused by a diverse mutation in a ATP7A ,copper transporting gene. Treatment with daily copper injections is useful in Menkes disease. Initial diagnosis of Menkes disease is supported by the demonstration of reduced levels of serum copper and ceruloplasmin. In neonatal period these markers should be interpreted carefully, as their levels are low in healthy newborns. Measurement of the neurochemical's dopamine, dihydroxyphenylacetic acid, norepinephrine, and dihydroxyphenylglycol in the plasma may be the choice as a rapid diagnostic test because of the low activity of dopamine-beta-hydroxylase, the ratio of dihydroxyphenylacetic acid to dihydroxyphenylglycol is distinctively increased in Menkes patient. High dopamine and norepinephrine ratio can be measured from catecholamine synthesis and metabolism(18). A study suggested that copper concentration in cultured fibroblast will significantly increase in cultured fibroblast from all patients than the control subjects. Another diagnostic proof of MD is the demonstration of the molecular defect in ATP7A(5). Radiographs of patients with classical MD show abnormalities due to copper deficiency. A light microscope of hair shows individual hairs that are twisted about their

own axes with varying shaft diameters and fragmentation at regular intervals(19). A study suggested that subcutaneous copper- histidine treatment has also been developed recently for 12 younger patients with good results. Another one suggested that parenteral copper-histidine supplementation may modify disease progression substantially, and the long term clinical outcome of four early-treated Menkes disease has been reviewed(20).

8. Conclusion

Menkes disease is a very rare X-linked recessive disease mainly resulting from a functionally impaired copper transport due to gene mutations in the ATP7A gene. The issue directly results in neurological worsening, neurodegeneration, and seizures, and impacts on connective tissues as well. A more moderate form of the disease is called Occipital Horn Syndrome (OHS) or Less-Serious Copper Deficiency and is also like Menkes disease, but not so serious. Although the disorder is prevalent in male subjects, females can also be infected through the rarity resulting from a unique gene reflection of X-chromosome inactivation or mutation. To date, ATP7A gene mutation has been confirmed to play a critical role in the development of Menkes disease; this is because of its defects in the transport of copper ions that leads to the above-mentioned disease symptoms. Experimental animals like mice genetically modified and other non-mammalian models like zebrafish and fruit flies have also been useful in fast-forwarding our understanding of disease progression and targeting therapeutic approaches. Diagnosis of menkes disease can be made by analyzing the level of copper and ceruloplasmin in the serum, neurochemical studies, and molecular study for the identification of the mutation in ATP7A gene. A primary diagnostic stage ensures early intervention that may be in the form of copper-histidine for changing the disease's progression. Radiographic and histologic changes, namely twirled hair shafts, corroborate this conclusion. About the future, the development in genetics and diagnostics makes a hope for better results in treatment and individualized therapeutic approaches for Menkes disease suffering persons. Save for the effective primary health care delivery for predetermined population groups, it is vital to mention the necessity of early diagnosis and integrated approach in treatment.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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