

MEDNIK syndrome: a new entry in the spectrum of inborn errors of copper metabolism

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*“There is nothing
Either
Good or bad
But thinking
Makes it so”*

William Shakespeare, *Hamlet, II, II*

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Copper homeostasis, including intestinal absorption, blood transport, uptake, trafficking, and excretion, is regulated by multiple genes encoding for specific copper transporters, which coordinate copper bioavailability [1]. Mutations in genes coding for copper pumps or copper chaperons are responsible for copper overload or deficiency, with relevant consequences on cell structure [2] and human health [3]. The spectrum of genetic disorders of copper metabolism includes multiple entities, characterized by different clinical presentations, with liver and brain as target organs. Wilson disease and Menkes disease are the most widely studied copper-related genetic disorders in humans. Wilson disease is an autosomal recessive disorder caused by a mutation in the *ATP7B* gene, encoding for a copper pump localized in the Trans-Golgi Network (TGN) or at the biliary pole of the hepatocyte, that is strongly expressed in the normal liver [4]. Malfunction or dislocation of *ATP7B* is at the basis of copper storage in the liver [5] and in the brain, in which copper overload is associated with storage of other trace elements such as iron [6]. Menkes disease, also known as kinky hair disease, is an X-linked, often fatal, neurodegenerative disease caused by a mutation in the *ATP7A* gene, encoding for another copper pump, *ATP7A*, whose malfunction is responsible for impaired copper absorption in the gut and decreased copper passage through the blood-brain barrier [7]. The Occipital Horn Syndrome is the milder variant of Menkes syndrome. Moreover, a distal motor neuropathy caused by a novel missense mutation in the *ATP7A* gene has been recently described [8].

In recent years, a new autosomal recessive mucocutaneous syndrome characterized by Mental retardation, Enteropathy, Deafness, peripheral Neuropathy, Ichthyosis, and Keratoderma (MEDNIK) has been described in several French-Canadian families. Previously defined as atypical erythrokeratoderma in 1972 [9], and as erythrodermia variabilis-3 in 2005 [10], in 2008 MEDNIK syndrome has been associated with a splice mutation in the Adaptor Protein-1 S1 (*AP1S1*) gene, encoding for the small subunit $\sigma 1A$ of the adaptor protein (AP)-1 complex [11]. This syndrome has been subsequently described in an Italian patient [12] and a Turkish child [13].

In its first report, MEDNIK syndrome was associated with a disarrangement of vesicular trafficking between organelles, ending with major changes in the development of skin and spinal cord, without any evidence of involvement of copper transport. An elegant study carried out in an Italian patient affected by MEDNIK syndrome revealed, for the first time, severe perturbations of

copper metabolism, including liver copper storage associated with intrahepatic cholestasis and low copper serum levels [12]. The similarities between MEDNIK syndrome and Wilson disease were confirmed by the finding in the same subject of low ceruloplasmin serum levels. On the other hand, studies in mutant fibroblasts carrying the same *AP1S1* mutations evidenced aberrant intracellular trafficking of *ATP7A* protein, revealing strong similarities between MEDNIK syndrome and Menkes disease. Thanks to the fundamental work of Martinelli and Dionisi-Vici, MEDNIK syndrome was included in the human defects of copper metabolism, with clinical and biochemical signs of both Wilson and Menkes disease [14]. Regarding the molecular pathways involved in MEDNIK syndrome, *AP1S1* has been identified as the pivotal gene involved in the correct functioning of the AP-1 complex, which is responsible for the crosstalk between the TGN and the other endosomes.

The discovery of MEDNIK syndrome highlights the role of AP complexes in copper metabolism. Indeed, as mentioned above, *AP1S1* encodes for the small $\sigma 1$ subunit of the AP-1, a member of the family of the heterotetrameric AP complexes. This family accounts for five members, from AP-1 to AP-5. Each complex consists of two large subunits (γ , α , δ , ϵ , ζ and $\beta 1$ - $\beta 5$), a medium subunit ($\mu 1$ - $\mu 5$), and a small subunit ($\sigma 1$ - $\sigma 5$). Therefore, the structures of the five adaptor protein complex are AP-1: γ , $\beta 1$, $\mu 1$, $\sigma 1$; AP-2: α , $\beta 2$, $\mu 2$, $\sigma 2$; AP-3: δ , $\beta 3$, $\mu 3$, $\sigma 3$; AP-4: ϵ , $\beta 4$, $\mu 4$, $\sigma 4$; AP-5: ζ , $\beta 5$, $\mu 5$, $\sigma 5$. Moreover, several adaptor protein complexes display different isoforms, depending on the different organs and tissue. For instance, two γ ($\gamma 1/\gamma 2$), and three σ ($\sigma 1A/\sigma 1B/\sigma 1C$) isoforms are known for AP-1, two α ($\alpha 1/\alpha 2$) isoforms for AP-2 and two β ($\beta 3A/\beta 3B$), two μ ($\mu 3A/\mu 3B$) and two σ ($\sigma 3A/\sigma 3B$) isoforms for AP-3 [14]. Two appendage domains (sometimes called ears) are linked by covalent chains to μ , and β subunits and the small σ subunit has a role in stabilizing the whole structure. AP-1, AP-3, and AP-4 all localize at the TGN or endosomes to facilitate protein sorting within the endosomal system. In contrast, AP-2 localizes at the plasma membrane to facilitate endocytosis. While the scaffolding proteins that facilitate AP-3 and AP-4 vesicle formation remain elusive, AP-1 and AP-2 complexes interact with clathrin and incorporate their cargos into clathrin-coated vesicles [15]. The sorting signals addressing these vesicles towards the proper target are typically small peptide motifs encoded in the cytoplasmic tail of transmembrane proteins. Although non-canonical

signals exist, basolateral sorting signals frequently conform to two types of sorting motifs: tyrosine-based motifs that conform to the YxxØ consensus sequence (in which Ø is a bulky hydrophobic amino acid F, I, L, M, or V), and di-leucine motifs often displaying [DE]xxxL[LI] sequences. Jains and colleagues have shown [16] that polarized sorting of the Cu²⁺ transporter ATP7B to the somatodendritic domain of rat hippocampal neurons is mediated by recognition of dileucine-based signals in the

cytosolic domains of the proteins by the σ1 subunit of the clathrin adaptor AP-1. Their studies suggest that in addition to failure to localize the TGN at low Cu²⁺ levels, the altered polarity of ATP7B (and perhaps also ATP7A) connected to variations of the small σ1 subunit of AP-1 in polarized cell types might underlie some of the copper metabolism defects in MEDNIK syndrome.

Fig. 1 shows the possible molecular connections between the *AP1S1* gene and the intracellular

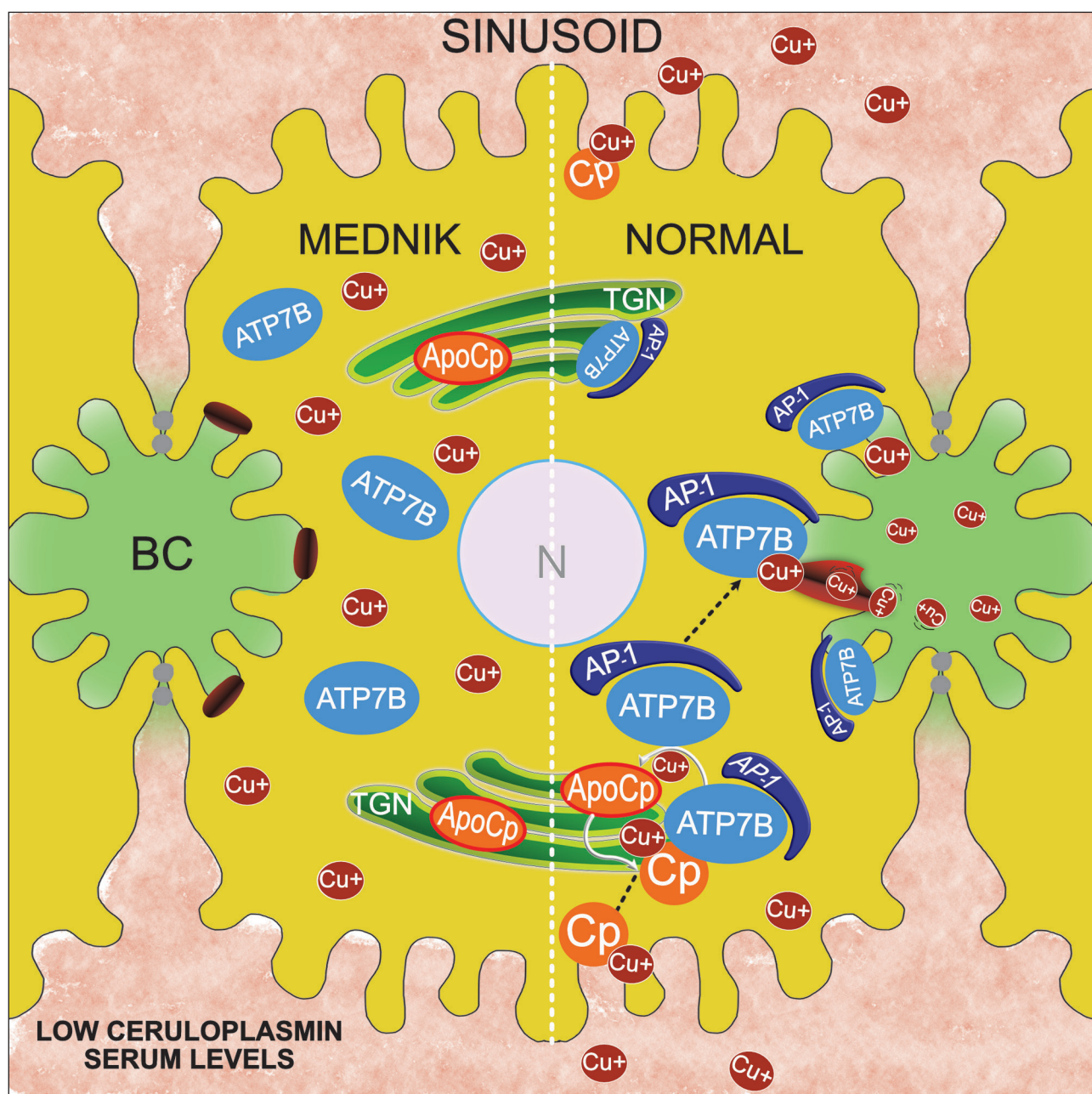


Figure 1. ATP7B trafficking inside hepatocytes.

On the right side, ATP7B in physiological conditions is mainly localized to the Trans-Golgi Network (TGN). When copper stores increase, ATP7B undergoes vesicular transport regulated by AP-1 and is transported to the biliary canaliculus (BC) membrane. In this location, ATP7B acts as a copper transporter, allowing excess copper excretion into bile. On the left side, the consequences of *AP1S1* gene mutations are summarised. The absence of AP-1 causes aberrant localisation of ATP7B, leading to copper accumulation in the hepatocyte and reduced incorporation of copper ions in apoceruloplasmin, ending with low ceruloplasmin serum levels.

trafficking of the copper pump ATP7B, playing a fundamental role in copper homeostasis.

The hypothesis that MEDNIK syndrome might represent the first example of diseases of copper metabolism associated with mutations in genes encoding for the subunits of AP complexes [14] has been recently confirmed by the report of three patients with a mutation in the *AP1B1* gene, encoding for the $\beta 1$ subunit of the AP-1 complex [17]. In these patients, a MEDNIK-like syndrome was observed, with low serum copper and ceruloplasmin levels, suggesting dysfunction of copper pumps, but in the absence of liver disease due to copper overload.

As all the cell machineries, copper pumps ATP7A and ATP7B need a correct vesicular transport, in order to be addressed towards their right subcellular compartment. MEDNIK syndrome evidences that a mutation in the *AP1S1* gene causes a perturbation of copper pump recruitment with subsequent heavy perturbation of copper metabolism. Given the role played by copper during development and, in particular, in the development of the nervous system, missense variations of the *AP1S1* gene may end with the impaired development of various neural networks, typical of the MEDNIK syndrome. The spinal cord, ending with ataxia and peripheral neuropathy, the central nervous system, leading to psychomotor retardation and intellectual disability and the inner ear, with sensorineural deafness, may be cited among them. Moreover, perturbation of the vesicular trafficking in all cells may explain skin and gut maldevelopment and liver disease due to copper overload.

MEDNIK syndrome opens a new field of human pathology, that has been called “adapinopathies” [14], including all congenital diseases associated with mutations in genes coding for adaptor complexes subunits. Moreover, the report of MEDNIK syndrome induces to focus on the developing spectrum of inborn errors of copper metabolism [18], where at least eight diseases may be included (**Tab. 1**). Nonetheless, the high number of copper chaperons involved in copper uptake and transport in human cells, including hCTR1, hCTR2, ATOX1, hCOX17, CCS, MURR1/COMMD1, induces to hypothesize an enlargement, in the next future, of this complex and fascinating field of human pathology. Deep knowledge of the molecular events at the basis of these diseases could ensure an early diagnosis in the perinatal period, allowing proper therapy and avoiding the insurgence of severe pathological complications in affected newborns.

Table 1. Spectrum of inborn errors of copper metabolism.

| Protein | Gene | Disease |
|---|------------------------------|---|
| Copper transporting ATPase 1 | <i>ATP7A (MC1, MNK)</i> | Menkes disease |
| | | Occipital Horn Syndrome |
| | | X-linked Distal Hereditary Motor Neuropathy |
| Copper transporting ATPase 2 | <i>ATP7B (PWD, WC1, WND)</i> | Wilson disease |
| Acetyl CoA transporter | <i>SLC33A1 (ACATN, AT1)</i> | Huppke-Brendel syndrome |
| Copper chaperone for superoxide dismutase | <i>CCS</i> | CCS deficiency |
| $\alpha 1$ subunit of the AP-1 complex | <i>AP1S1 (AP19, CLAPS1)</i> | MEDNIK syndrome |
| $\beta 1$ subunit of the AP-1 complex | <i>AP1B1 (ADTB1, CLAPB2)</i> | MEDNIK-like syndrome |

Declaration of interest

The Authors declare that there is no conflict of interest.

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