

**METAL DYSHOMEOSTASIS IN THE ETIOLOGY OF BILIRUBIN-INDUCED  
NEUROLOGIC DYSFUNCTION (D-PENICILLAMINE AS AN  
ANTIRETROVIRAL DRUG IN HIV OR EBOLA INFECTION DUE TO VERTICAL  
TRANSMISSION)**

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**ABSTRACT** *AIM: The aim of this review was to demonstrate a new concept in the etiology of bilirubin-induced neurologic dysfunction (BIND) and highlight the role of D-Penicillamine (D-PA) in the treatment of HIV or EBOLA infection due to vertical transmission. METHOD: We conducted a review searching the literature of bilirubin metabolism and of metal-homeostasis, furthermore of neonatal HIV and EBOLA infection. RESULTS: Over the past two decades there have been significant advances in our understanding of copper homeostasis and of neurodegenerative and neurodevelopmental diseases (NDs), and the pathological consequences of copper dysregulation. Thus, comprehension of metal homeostasis, details of transport and interactions with biomolecules, such as unconjugated bilirubin (UCB) or albumin, is essential for understanding the normal and pathological processes occurring in the neonatal period. UCB has a special affinity for the globus pallidus, the hippocampus, and the subthalamic nucleus. Furthermore, immaturity of the blood-brain barrier (BBB) also plays a role in kernicterus. Homeostasis of metal ions usually involves a huge set of proteins which regulate the proper metal biology. Metal ions, especially copper and iron play very important roles in NDs including BIND, having impact on both protein structure (misfolding) and oxidative stress. INTERPRETATION: Free copper ion in itself or binding to UCB and forming metal-bilirubin complex(es) involved in neurologic dysfunction; therefore they are important factors for central nervous system (CNS) damage processes in BIND by the production of free radicals. Our present research article address the medical necessity of the use of a chelating agent (D-PA) in the treatment of neonatal hyperbilirubinemia (NHBI). Finally, the authors highlight that D-PA may have a huge impact on HIV or EBOLA infection caused by vertical transmission where NHBI is a very common symptom.*

**KEYWORDS:** Bilirubin-Induced Neurologic Dysfunction; Reactive Oxygen Species; Copper Dyshomeostasis; HIV or EBOLA infection; Neurodegeneration; D-Penicillamine in the Neonatal Period

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## **INTRODUCTION**

The classic form of chronic bilirubin encephalopathy (kernicterus) is a well-described clinical tetrad of ► abnormal movements and muscle tone, ► an auditory processing disturbance with or without hearing loss, ► oculomotor impairments, especially impairment of upward vertical gaze, and ► dysplasia of the enamel of deciduous teeth. Hervieux first described the condition in 1847, and Schmorl first used the term kernicterus as early as 1903.<sup>1,2</sup> NHBI is a common condition in the first week of postnatal life. Although generally harmless, some neonates may develop very high levels of UCB. Subtle encephalopathy or BIND refers to individuals with

subtle NDs.<sup>3-5</sup> In the past decades, interest in bilirubin damage of the brain has been reawakened by an increase in its prevalence, owing to failure to closely observe infants discharged from the hospital well before the peak of NHBI.<sup>6</sup> There is a tremendous variability in babies' vulnerability toward UCB for reasons not yet explained, but preterm birth, sepsis, hypoxia, hypoperfusion, hyperosmolality, acidosis, hypalbuminemia and *hemolytic disease et cet.* (underlying diseases or comorbidities) are comprised as risk factors, so, the UCB levels and neurological abnormalities are not strictly correlated. Kernicterus may only be the "tip of the iceberg." Subtle UCB damage may account for many more cases of: learning disabilities, central auditory processing disorders, dyslexia, oculomotor dyspraxia, movement disorders, and autism spectrum disease (ASD), and may even predispose to Parkinson's disease (PD) or schizophrenia in adulthood.<sup>7</sup> The pathomechanisms of BIND have not been fully understood yet. The mechanisms of UCB neurotoxicity are still also unclear, and little is known about lasting sequelae attributable to NHBI. Our hypothesis addresses the medical necessity of chelation therapy (with D-Penicillamine – D-PA) in the neonatal period<sup>8,9</sup> as it is feasible that UCB molecule reveals particular affinity to copper stored in basal ganglia (BG) of the neonatal brain, where copper-bilirubin complex can be formed together with the production of hydroxyl radical (OH<sup>-</sup>). In addition, various amount of free metal ions can be found in the intravascular space and in the tissues (especially in BG) during *hemolytic processes*.

### **Pathological basal ganglia activity**<sup>10</sup>

The BG is a collection of large subcortical nuclear masses. It is agreed that core components comprise the caudate nucleus, the nucleus accumbens, the putamen, and the globus pallidus. The caudate nucleus and putamen together are sometimes called the striatum, and the putamen and globus pallidus are together sometimes described as the lentiform nucleus.<sup>11, 12</sup> Functionally, the BG has considerable connections to the cerebral cortex, thalamus, and brain stem; so, anatomists consider portions of the thalamus as components of the BG.<sup>13</sup> A literature review was aimed at assisting us (as pediatricians) to provide further understanding with bilateral symmetrical BG and thalamic lesions on magnetic resonance imaging (MRI). The high-signal-intensity lesions on T<sub>2</sub>-weighted images can be caused by edema, gliosis, demyelination, neuronal necrosis, or cystic degeneration both in Wilson disease (WD) and BIND.<sup>14</sup>

### **Role of metals and oxidative stress in the human neurodegenerative and neurodevelopmental disorders**

The brain (mostly the BG) accumulates among the highest levels of transition metals in the body for normal function, including redox-active copper. This high-redox metal load, in combination with the brain disproportionately active oxygen metabolism, makes this organ particularly susceptible to oxidative stress.<sup>15-18</sup> Metal ions such as calcium, zinc, iron and copper are key players in brain neurobiology; their homeostasis is altered in most ND conditions. The **metal dyshomeostasis (MD)** in the brain and related organs, and **loss of the strict regulation** is implicated in neurotoxic stress<sup>19-21</sup> and in a variety of NDs including BIND and prion-mediated encephalopathies and other diseases<sup>22-24</sup> which can be seen in the **Table 1.**<sup>25-47</sup> Parts emphasized with **bold-faces** show associations between the ND illnesses and BIND. Pathologic changes to the CNS in these disorders are always associated with a significant dyshomeostasis of tissue metals (particularly *copper*). Excess copper may combine with sulfhydryl, carboxyl, or amine groups, resulting in improper enzymatic activity or damage to cellular structure. Despite the ubiquitous presence of toxic copper within the brain, pathologic findings are limited primarily to the BG, thalamus, and brain stem. Histopathologic

studies have shown abnormalities throughout this system in patients suffering from MD. These abnormalities include atrophy, spongy softening, cavitation, a general reduction of neurons, increased cellularity, and the presence of characteristic cells (Opalski, Lewy bodies). The pathologic changes are presumed to result from an increased amount of extracellular copper, which causes oxidative stress and results in cell destruction.<sup>48</sup> Many diseases of the BG have some *disorder of movement* as their primary symptom, ranging from an excess of (abnormal) involuntary movements such as in chorea to a poverty and slowness of movement as in PD, Alzheimer disease (AD) and WD as illustrated in several clinical cases<sup>49</sup> and **UCB encephalopathy**<sup>50</sup> where a characteristic yellow staining can be observed in fresh or frozen sections of the brain obtained within 7-10 days after the initial bilirubin insult. If the affected infant survives the neonatal period and subsequently dies, the yellow staining may no longer be present, but the BG will display microscopic evidence of cell injury, neuronal loss, and glial replacement. Newborns, especially preterm infants, are particularly vulnerable to reactive oxygen species (ROS) because they exhibit accelerated production of free radical and limited antioxidant protection, which increases the susceptibility of rapidly growing tissues to damage. „Free radical-related diseases” of neonates promote cellular, tissue, and organ impairments. In 1988, Saugstad coined the phrase „oxygen radical disease in neonatology” to highlight the crucial role of ROS in a wide range of neonatal disorders.<sup>51</sup> There is now a large body of literature demonstrating that free or weakly bound iron and copper ions may exert their toxic action on BG. In a way, metals may provide the link between protein misfold and aggregation, oxidative stress and the cascade of biochemical alterations, eventually leading to neuronal cell death. Predominantly the cellular content of copper determines copper-induced toxicity in brain astrocytes.<sup>52</sup>

### **Potential molecular mechanisms of bilirubin-induced neurologic dysfunction**

The “classic” interpretation of bilirubin neurotoxicity does not give sufficient answers to the following questions: (1) How to call bilirubin: friend or foe? (2) If the bilirubin is really an „enemy”, how does it induce its dangerous effects?

#### **Ad (1)**

The exact UCB concentration associated with kernicterus in the healthy term infant is unpredictable. In a Danish population-based study, the neonates with total serum bilirubin levels of  $\geq 25$  mg/dL didn't show any neurologic dysfunctions at 5 years of follow up.<sup>53</sup> Toxicity levels may vary among ethnic groups, with maturation of an infant, and mainly in the presence of *hemolytic disease*. Bilirubin, which is derived from its metabolic precursor biliverdin, is the end product of heme catabolism. It has been proposed that UCB is an excellent endogenous antioxidant present in human extracellular fluids<sup>54</sup>. Bilirubin can suppress oxidation of lysosomes at oxygen concentrations that are physiologically relevant. It can act as an important cytoprotector of tissues that are poorly equipped with antioxidant defense systems, including myocardium and nervous tissue.<sup>55, 56</sup> Serum bilirubin in jaundiced and non-jaundiced pups exposed to 95% O<sub>2</sub> shows a negative correlation with lipid hydroperoxides at 3 days of exposure. Higher UCB concentrations resulted in lower lipid hydroperoxide levels.<sup>57</sup> Therefore, we think that UCB in itself is actually our friend, that is: Bilirubin, The Gold Within.<sup>58</sup> (BILIRUBIN- FRIEND OR FOE?? :

Function as natural antioxidants in newborns. Attenuates graft rejection in cardiac transplant models. Inverse relation between bilirubin and coronary artery disease. Inverse relation

between bilirubin and colorectal cancer. – 2005 Powerpoint Presentation  
[www.sfrbm.org/frs/FrielBilirubin.pdf](http://www.sfrbm.org/frs/FrielBilirubin.pdf) )

## Ad (2) Toxic Side of Bilirubin

Erythrocyte morphological changes have been seen with incubation of cells with different molar ratios of UCB. These changes occur as the bilirubin/human serum albumin molar ratio increases. This indicates that bilirubin can illicit toxicity in the erythrocyte membrane in a concentration and temperature-dependent manner, causing *hemolysis*.<sup>59</sup> Several studies have found that NHBI is associated with higher risk of movement disorder and, even more, developmental delay. The management dilemma for a clinician is that UCB is a beneficial antioxidant at low (and may be at moderately higher) levels, but a neurotoxin at >20 mg/dL levels (“vigintophobia”<sup>60</sup>), where it can impair the normal developmental maturation of the neonatal brain.

Among the 23 elements with known physiological functions, 12 are metals (sodium, magnesium, potassium, calcium, vanadium, chromium, manganese, iron, cobalt, copper, zinc, and molybdenum).<sup>61, 62</sup> Copper is essential for the normal growth and development of human fetuses, infants, and children and it is crucial for the normal development of the brain<sup>63</sup> which has among the highest levels of copper, as well as iron and zinc, in the body. *Copper* is an interesting essential micronutrient. *Deficiency and excess intake both induce a variety of clinical manifestations* affecting mainly the hematopoietic system, the skeleton, the liver, and the brain. Although copper transport to the fetus is high and liver storage is efficient, copper export from the hepatocytes to the bile and to blood ceruloplasmin (Cp) are reduced during this stage of life because of liver function immaturity. This leads to a high copper accumulation in the liver and brain, in a magnitude *similar to that observed in Wilson disease*. In fact, an obvious analogy can be observed between the *newborns and patients with Wilson disease* in the field of the copper „(dys)homeostasis” (see: **Table 1**). The increased liver and brain copper storage of the fetus may have a selective evolutionary advantage since it may prevent copper deficiency during the first months of life when the child receives a relatively low copper supply from breast milk.<sup>64</sup>

## Metal regulatory proteins in the neonatal period

A variety of **proteins** are involved in the regulation of metal metabolism and the oxidative response and many are involved in iron or copper metabolism due to the redox activity of those metals. Protein misfolding and conformational changes are also a cornerstone of NDs. All metals with known physiological functions are bound by **albumin**.<sup>65, 66</sup> A decrease in metal binding of albumin means *more free metal available to produce oxidative stress* and other physiological effects such as influence of calcium (Ca<sup>++</sup>) homeostasis by altering the conformational structure of the pumps, enzymes, binding proteins, and channels that regulate Ca<sup>++</sup> flow. Often, this results in elevating free intracellular Ca<sup>++</sup> levels which may produce depletion of glutathione/GSH with a downstream induction of DNA damage and eventual cell death.<sup>67</sup> Therefore, the bilirubin-mediated neurotoxicity is partly due to increased rate of cell apoptosis and *higher levels of intracellular free Ca<sup>++</sup> ion* level (as analogy, see **Table 1**: Fahr’s disease).<sup>42</sup>

**Ceruloplasmin (Cp)** is a large blood protein synthesized by the liver with the primary role of transporting copper. If a disease process (e.g. hepatic failure) or *insufficient synthesis in the neonatal period* lowers the production of Cp, the free copper would increase and copper

mediated oxidative stress would be enhanced. In addition, there is some evidence that under oxidative stress conditions, Cp may induce further oxidative stress in a manner akin to a positive feedback mechanism. Also, when this protein is exposed to ROS, its ability to bind copper is reduced, releasing free copper, producing further oxidative stress.<sup>68</sup>

**Copper transporter 1 (Ctr1)** has a high affinity for copper and serves to transport copper into the interior of the cell. It is not highly expressed in the brain, where the choroid plexus may contain the greater proportion. The lower levels of expression in the brain, however, should not be taken as a sign that Cu metabolism is not important in the brain as several neural pathologies (Alzheimer's disease, spongiform encephalopathies) have been linked to disordered copper metabolism.<sup>69</sup>

**Metallothionein (MT)** is a cysteine rich protein involved in the regulation of zinc and other metals (mainly copper, and selenium). This protein is found in a variety of forms (I-IV) in mammals, and MT<sub>II</sub> are the most abundant in the CNS where MT is found mostly in astrocytes. MT plays an important role in cell signaling. Neonatal brain has lower MT concentrations than adult brain, increasing to adult levels by Day 21.<sup>70</sup> Elucidating the role of the **metallochaperone Atox1**,<sup>71, 72</sup> it is obvious that Atox1-deficient cells accumulates high levels of intracellular copper, and metabolic studies indicate: this defect originated from the impaired cellular copper efflux. These data reveal a direct role for Atox1 in trafficking of intracellular copper to the secretory pathway of mammalian cells and demonstrate that metallochaperone plays a critical role in perinatal copper homeostasis. To sum up, the number of proteins involved in metal oxidative stress is large and fall into two groups: those involved in iron or copper metabolism, and those involved with the rest of the metals. Bilirubin-metal complexes have been made in vitro producing hydroxyl radical ( $\text{OH}^-$ ) and there are no reasons not to believe that such complexes can also exist in vivo, especially since UCB can take on a ring-like configuration with one of the end pyrrol-rings in a lactim form and the other in a lactam form.<sup>73, 74</sup> To clarify how hyperbilirubinemia influences neurodevelopmental outcome, more sophisticated consideration is needed both of how to assess bilirubin exposure leading to neurotoxicity and of those comorbid conditions which may lower the threshold for brain injury.<sup>46</sup> A decrease in metal binding means of course more free metal available to produce oxidative stress and other physiological effects. Six substances are transported with albumin: long chain fatty acids *bilirubin*, steroids, thyroid hormones, drugs and *copper* (also other metals eg. Zn, Pb...).<sup>75</sup> Albumin interacts with a broad spectrum of compounds. Most strongly bound are hydrophobic organic anions of medium size, 100 to 600 Da—long-chain fatty acids, hematin, and bilirubin. The equilibrium constant of UCB is about  $3.8 \pm 2.0 \times 10^7 \text{ M}^{-1}$  and the calculated Cu ion binding constant is  $1.50 \times 10^6 \text{ M}^{-1}$ . In comparison, albumin is interacting selectively and non-covalently with Cu ions.<sup>76-79</sup> Neonatal blood has low content of glutathione peroxidase, superoxide dismutase,  $\beta$ -carotene, riboflavin,  $\alpha$ -proteinase, vitamin E, selenium, copper, zinc, ceruloplasmin and other plasma factors. The premature brain is rich in polyunsaturated fatty acids, and easily oxidized compared to monounsaturated fatty acids.<sup>80</sup>

### **New concept for development of bilirubin-induced neurologic dysfunction**

**Table 1.** illustrates well that a remarkable similarity exists between Wilson disease and BIND. Very wide-ranging studies have long been made on the possible biochemical transformations of UCB, which is formed during the decomposition of haemoglobin. Particular attention has been paid to its photochemical and redox reactions<sup>81</sup> but the relevant publications comprise only a very small proportion of those dealing with the molecular biochemistry of *UCB and*

*metals interactions*. Bilirubin has a special affinity for the globus pallidus, the hippocampus, and the subthalamic nucleus because they are also target brain regions for divalent metal (Cu, Fe, Zn et cet.) accumulation.<sup>82</sup>

**Neurodegeneration: a return to immaturity?**<sup>83</sup> This question certainly arouses the attention of neonatologists as *the immature and strikingly vulnerable neurons* play important role in the pathogenesis of BIND. The increased vulnerability of premature infants to brain damage may be due to a proneness of immature nerve cells to toxic stimulus. The developing neurons undergo programmed cell death, a necessary phenomenon for proper nervous system development. Following the developmental period, neurons mature and restrict the apoptotic pathway to permit long-term survival. On the basis of above described abundant research data and hypotheses, **according to our concept, the BIND is an ND of immature brain caused by accumulation of free metals and UCB-Cu complex (as prooxidant) in the BG and other parts of CNS relevant to BIND.** The rate of formation of UCB-Cu complex when bilirubin extracts copper from copper–albumin complex, as obtained in a very exciting experiment, is  $34.98 \text{ l mol}^{-1} \text{ s}^{-1}$ <sup>84</sup>. The main comorbidity is the **hemolysis** of neonatal blood red cells.<sup>85</sup> During this process a great amount of heavy metals (mainly iron and copper) may circulate in free form in the bloodstream, and can pass through the BBB, finding entrance into the CNS as well. Understanding the differences between neonatal and adult erythrocytes is critical in the evaluation of perinatal erythrocyte disorders. The reason for the reduced RBC survival observed in newborns is not known, although there are many biochemical differences between adult and neonatal RBCs.<sup>86</sup> Increased oxidant sensitivity of newborn red cells and relative instability of fetal hemoglobin have been considered as possible causes for this shortened lifespan. In a chinese study,<sup>87</sup> the erythrocyte's copper content was significantly lower in the maternal blood than in the newborn cord blood. The compounds to be bound and transported by albumin are quite diverse and include bilirubin, fatty acids, metal ions and therapeutic agents. Bilirubin itself can displace metals (copper) from the albumin binding because UCB binds stronger to albumin than copper, in other words, **copper loosely bound to albumin.**<sup>88</sup> Free or loosely bound, redox-active transition metal ions are potentially extremely pro-oxidant, having the ability to catalyze the formation of damaging and aggressive ROS from much more innocuous organic and inorganic species. In strictly biological terms the two most important such metals are iron and copper.<sup>89</sup> In fact, oxidative stress has been demonstrated to be a common link between several conditions such as PD, AD, stroke, prion diseases and UCB encephalopathy, where it is involved in neuronal injury.

### **D-penicillamine as neonatal neuroprotectant**<sup>90,91</sup>

Our recently published case reports<sup>92</sup> and other healthy and highly educated patients of the long-term (28-42 years) follow-up suggest that D-PA therapy of newborn infants may have significant neuroprotective effects in cases jeopardized by BIND or ROP. The first patient (42 ys) is now a member of a famous operahouse in Germany as an opera singer, the second one (16 ys) is excellent in music and matematics. These cases are all the more remarkable as the most common sequelae of NHBI is the sensorineural hearing impairment. These unexpected effects may be related to DPA capability to alter the most important gasotransmitters (nitric oxide /NO/ system, carbon monoxide /CO/, hydrogen sulfide /H<sub>2</sub>S/ biosynthesis, and Cu<sup>++</sup> homeostasis in the brain). According to our hypothesis DPA can modulate the function of these neurotransmitters and can protect the brain (especially the BG and retina) from injury, such as BIND and ROP. D-PA not only chelates copper from tissue, but also detoxifies tissue copper by promoting the synthesis of metallothionein, which forms a non-toxic combination with copper.<sup>36</sup> There are very important **age-related effects of D-PA in the neonatal period.**

In the **Table 2**, we demonstrate the results of our animal experiments regarding the age related differences in effects of D-PA.<sup>93</sup> The high activity of heme oxygenase in the newborn could reflect the enzyme-inducing action of metals: Cu and Fe derived from the breakdown of fetal erythrocytes. Chelation therapy in neonates restores the normal activity of enzymes participating in heme metabolism. Briefly, chelating agents facilitate heme synthesis and inhibit heme degradation. In other words, DPA as a chelating agent, boost or inhibit the immature enzyme systems to the adult levels. The main comorbidity is the *hemolysis* of neonatal blood red cells.<sup>85</sup> During this process a great amount of heavy metals (mainly iron and copper) may circulate in free form in the bloodstream, and can pass through the BBB, finding entrance into the CNS as well.<sup>94</sup>

## CONCLUSION

The basic role of metal ions in neurological pathologies is generally accepted, — except for the case of BIND. Free copper ion in itself or binding to UCB and forming metal-bilirubin complex(es) involved in neurologic dysfunction, therefore they are important factors for whole brain damage processes in BIND. **Figure 1**, demonstrates our concept about the chronic bilirubin encephalopathy based on the above described hypothesis. We hope that our theory will help answer some of the unsolved questions and concerns occurred in the etiology and pathomechanisms of BIND. The beneficial neuropharmacological actions of metal-targeted (chelating) agents most likely arise from local metal redistribution rather than from massive metal removal.<sup>95</sup> The chelation therapy for non-metal overload indications continues to be investigated. Our present research article address the medical necessity of the use of a chelating agent (D-PA) in the treatment of NHBI.<sup>96-106</sup>

### **Possible beneficial effects of D-PA on the lethality of HIV or EBOLA infection due to vertical transmission**

West Africa is currently in the midst of the largest EBOLA outbreak in the history and HIV prevalence in sub saharan Africa is also very high.

The structural and functional properties of DPA make it suitable for exerting antiviral activity. This drug caused a marked inhibition of polyo-virus-specific RNA and the protein synthesis.<sup>108</sup> Searching the pertinent literature, several publications relating to the beneficial effects of D-PA-therapy in the treatment of AIDS-patients were found. The high doses resulted in good outcomes, but adult patients did not tolerate this therapy. In addition to this, it has been determined that the selective inhibition of replication of HIV type 1 (further: HIV) by this drug was concentration dependent, that is, at 40 microgram/ml concentration D-PA completely inhibited HIV replication in H9 cells in vitro<sup>109</sup> (*a single 100 mg/kg bw. IV administered DPA resulted in more multiple plasma concentration in premature infants*<sup>110</sup>). This study has a promising idea wondering whether or not it is true that DPA has possible beneficial effects on the AIDS or EBOLA associated infant mortality rates because of its prolonged antiviral activity. Abundant experimental evidence and clinical observations exist to suggest that early viremia and immune responses in vertical HIV infection are different from those of adults. The developing immune system might allow for more efficient viral replication and less efficient immune containment of viral replication. In this respect, D-PA-therapy may be a potent early regime to control HIV (or EBOLA) replication and offers the golden opportunity to prevent or reverse the rapid progression of these diseases. The potential

mechanism of antiretroviral actions of D-PA in infections caused by vertical transmission are as follows:

- It is presumed that antioxidant treatment (D-PA is a well-known direct antioxidant) may provide a promising and cost-effective therapeutic approach in treating neonatal HIV or EBOLA infection. The newborn infants, especially the prematures, are suffering in an oxidative stress condition <sup>111</sup>
- It acts as a potent protease inhibitor in animal model <sup>112</sup>.
- The copper metabolism in Wilson's disease and in newborn infants is strikingly similar: they both have large quantities of copper in the liver and low ceruloplasmin in the blood. It was previously found that cupric chloride, in the presence of a chelating agent, could inhibit the HIV-1 protease <sup>113</sup>.
- Extra cystein given in the form of DPA ( $\beta$ - $\beta$ -dimethylcysteine) can cause an increase in intracellular cysteine and glutation content which play an important role as HIV inhibitors, at least in part because they facilitate the intracellular transport of Zn and Cuions <sup>114</sup>.
- The HIV-1 nucleocapsid p7 protein contains two retrovirus-type zinc finger domains that are required for multiple phases of viral replication. Considering the chelating properties of DPA and its disulfide reaction with cysteine, one can conclude that HIV or EBOLA-replication could be inhibited by this drug <sup>115-120</sup>.

It would be very exciting to be involved in this work, especially that D-PA will have a huge impact on HIV or EBOLA infection caused by vertical transmission *where NHBI is a very common symptom*.

### Conflict of Interest Statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

### Abbreviations

AD - Alzheimer disease; ASD - autism spectrum disease; BBB - Blood brain barrier; BG - basal ganglia; BIND - Bilirubin-induced neurologic dysfunction; CNS- central nervous system; Cp - ceruloplasmin; CuFr - Free copper ion; D-PA - D-Penicillamine; MD - Metal dyshomeostasis; MRI - Magnetic resonance imaging; MT - Metallothionein; NHBI - Neonatal hyperbilirubinemia; OH<sup>-</sup> - Hydroxyl radical; NDs - Neurodegenerative and neurodevelopmental diseases; PD - Parkinson disease; ROS - Reactive oxygen species; UCB - Unconjugated bilirubin; UCBFr - free UCB; WD - Wilson disease

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**Table 1.** Neurodegenerative and neurodevelopment diseases involving metal dyshomeostasis (interpretation can be found in the text)

<b>Disease</b>	<b>Clinical symptoms</b>	<b>Histopathology <i>MRI</i></b>	<b>Metal dyshomeostasis</b>
Aceruloplasminemia <sup>25</sup>	<b>Ataxia, chorea,</b> blepharospasmus, dementia	<b>Nerve cell loss,</b> <b>abnormal mastocytes, glubular structure;</b> <i>Bilateral low signal on T2 at the basal ganglia</i>	Redox active <b>iron</b> <b>accumulation</b>
Alzheimer disease <sup>26-28</sup>	Dementia and <b>movement disorders</b>	Neuronal and synaptic loss, amyloid plaques, <b>neurofibrillar degeneration;</b> <i>Hippocampal and whole brain atrophy</i>	<b>Abnormal accumulation</b> and distribution of reactive iron and <b>copper</b>
Amyotrophic lateral sclerosis <sup>29</sup>	Slowly progressive, <b>painless weakness,</b> with limb involvement or bulbar symptoms	Cerebral atrophy, <b>upper motor neuron dysfunction</b> <i>Hyperintense corticospinal tracts</i>	<b>Copper mediated</b> neurological disorder
Menkes's disease <sup>30</sup>	Brittle, kinky hair Feeding difficulties Irritability <b>Lack of muscle tone, floppiness (hypotonia)</b>	Extensive <b>neurodegeneration</b> int he gray matter and arteries of the brain. <i>Cerebral and cerebellar atrophy, delayed myelination, bilateral basal ganglia changes (thalamus)</i>	<b>Copper mediated</b> neurological disorder
Huntington disease <sup>31</sup>	<b>Impairments in voluntary movements</b> and cognitive function, behavioral and pszichiatric disorders	<b>The striatum is the area</b> most severely impacted; the outer cortical regions also shows damage <b>T2 ypointensities in basal ganglia</b>	Mitochondrial <b>metal dyshomeostasis</b> and dysfunction Copper and iron concentrations are increased in the striata

Occipital horn syndrome <sup>32</sup>	Soft skin, <b>weak muscle tone</b> , stroke, internal bleeding, kyphoscoliosis	A rare disorder of elastic tissue resulting in loose, redundant, hypoelastic skin. <i>Irregularities consistent with periventricular leukomalacia</i>	Inborn errors of metal ( <b>copper</b> ) <b>metabolism</b>
<b>Disease</b>	<b>Clinical symptoms</b>	<b>Histopathology</b> <b>MRI</b>	<b>Metal dyshomeostasis</b>
Parkinson disease <sup>33-35</sup>	<b>Tremor (shaking)</b> <b>Slowness of movement</b> Rigidity (stiffness) Sleep problems Anxiety, Dementia Depression	Loss of dopaminergic neurons from the <b>substantia nigra</b> associated with the presence of intraneuronal inclusions called Lewy bodies. <i>With new imaging techniques: diffusional changes in the orbital-frontal region in the pre-motor phase of PD</i>	<b>Iron</b> accumulation and <b>dyshomeostasis</b>
Wilson disease <sup>36, 37</sup>	Deterioration in school performance or handwriting, mild <b>tremors, dystonia, ataxia</b> , muscular rigidity, <b>dysarthria</b>	<b>The lenticular nuclei</b> macroscopically appear brown in color, <b>degeneration</b> occurs with disease progression, leading to necrosis, gliosis and cystic changes, and lesions can be seen in the brainstem, thalamus, cerebellum and cerebral cortex, presence of Opalski cells. <i>Hyperintense signals in putamen and globus pallidus</i>	<b>Copper accumulation in the basal ganglia</b>
Distonias <sup>38</sup>	Dystonia is an undesirable, <b>involuntary muscular movement</b> of a part of the body.	Related to a problem <b>in the basal ganglia</b> . <i>In most cases, no abnormalities are visible using MRI or other diagnostic imaging.</i>	Detrimental effects of transition metal ( <b>copper</b> ) <b>dysregulation</b>
Tourette syndrome <sup>39</sup>	<b>Different types of tics:</b> arm thrusting, eye blinking, jumping, kicking, repeated throat clearing or sniffing, shoulder shrugging	Structural changes in frontal cortex and <b>striatum</b> <i>Structural changes in the basal ganglia, limbic structures and prefrontal cortex, T2 relaxation time asymmetries</i>	Genetic heavy metal toxicity, Free <b>copper</b> may be <b>elevated</b>
Autism spectrum disorders (ASD) <sup>40, 41</sup>	Delayed speech development,	The cerebellum have documented cellular and neurochemical	The Zn/Cu ratio could be considered as biomarker of

	<p>reacting unusually negatively, having <b>repetitive movements</b>. Developmental disorder affecting people interact with the world.</p>	<p>alterations, cellular and gross anatomic changes of the vermis cerebelli <i>Abnormally increased total brain volume.</i> <i>Decreases in cortical thickness in the frontal, temporal, and occipital lobes.</i></p>	<p>ASD. Free <b>copper</b> may be <b>elevated</b></p>
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Disease	Clinical symptoms	Histopathology <i>MRI</i>	Metal dyshomeostasis
Fahr's disease <sup>42</sup> IBSPDC.	<p><b>Gait</b> and speech <b>disturbance</b>, limb and truncal <b>ataxia</b>, dementia, <b>bradykinesia</b>, <b>mild parkinsonism</b></p>	<p>Idiopathic bilateral <b>striatopallidodentate calcinosis</b> (IBSPDC) <i>A striking high density area in the basal ganglia and the dentate nuclei of the cerebellum.</i></p>	<p>The <b>increased levels of Cu, Zn, Fe</b> and Mg reflect the involvement of metabolism of several metals and/or metal-binding proteins during the progression of IBSPDC. <b>Neurodegeneration with brain iron accumulation</b></p>
Hallervorden-Spatz (HSD) disease <sup>43</sup>	<p>Progressive dementia, <b>spasticity</b>, <b>rigidity</b>, <b>dystonia</b>, and <b>choreoathetosis</b></p>	<p>Juvenile-onset generalized <b>neuroaxonal dystrophy</b> <i>Bilaterally symmetrical, hyperintense signal changes in the anterior medial globus pallidus, with surrounding hypointensity in the globus pallidus, on T2-weighted images.</i></p>	
<b>BIND</b> <sup>44-47</sup>	<p>Neurological, learning and movement disorders, isolated hearing loss, and auditory dysfunction</p>	<p>The abnormalities result from damage to the basal ganglia. <i>High T2 signal in globus pallidus</i></p>	<p>„Abnormal” accumulation and distribution of reactive iron, <i>copper</i> and zinc in the basal ganglia</p>

**Table 2. Age-related differences in the effects of D-Penicillamine**

	Neonates	Adults
<b>Hexobarbital sleeping-time</b>	<b>shortened</b>	<b>no effect</b>
<b>Hem-oxygenase</b>	<b>inhibited</b>	<b>no effect</b>
<b>Cytochrom- P- 450</b>	<b>increased</b>	<b>no effect</b>
<b>Catalase</b>	<b>increased</b>	<b>no effect</b>
<b>Peroxidases</b>	<b>increased</b>	<b>no effect</b>
<b>LD<sub>50</sub></b>	<b>&gt; 4000 MG/KG (IP)</b>	<b>500 MG/KG (IV)</b>
<b>Radioprotection</b>	<b>significant</b>	<b>?</b>

**Figure 1**

Potential pathomechanisms of bilirubin-induced (metal mediated) neurodevelopmental dysfunction (BIND), and effects of D-Penicillamine (D-PA) in the neonatal hyperbilirubinemia (ROS = reactive oxygen species; CuFr = free copper; UCBFr = free bilirubin). Interpretation of D-PA written sideways: D = direct antioxidant; | = scavenge of ROS and chelation of metals; P = inhibition of heme oxygenase (a decreased production of UCB) only in the newborn period (age related effect); A = copper chelation

