Metallomics

CRITICAL REVIEW

Characterization of biometal profiles in neurological disorders†

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Many neurodegenerative and neuropsychiatric disorders have been reported to coincide with the dysregulation of metal ions in the body and central nervous system. However, in most cases, it is not the imbalance of a single divalent metal ion but a plethora of metal ions reported to be altered. Given that different divalent metal ions are often able to bind to a protein in a competitive manner, although with different affinities, and that they might use similar transporters for uptake and regulation, it is likely that the imbalance of one metal ion will downstream affect the homeostasis of other metal ions. Thus, based on this assumption, we hypothesize that the dysregulation of a specific metal ion will lead to a characteristic biometal profile. Similar profiles might therefore be detected in various neurological disorders. Moreover, if such shared biometal profiles exist across different neurological disorders, it is possible that shared behavioural impairments in these disorders result from the imbalance in metal ion homeostasis. Thus, here, we evaluate the reported excess or deficiency of metal ions in various neurological disorders and aim to integrate reported alterations in metal ions to generate a characteristic biometal profile for the disorder. Based on this, we try to predict which alterations in biometals will be caused by the overload or deficiency of one particular metal ion. Moreover, investigating the behavioural phenotypes of rodent models suffering from alterations in biometals, we assess whether a shared behavioural phenotype exists for disorders with similar biometal profiles. Our results show that observed behavioural aspects of some neurological disorders are reflected in their specific biometal profile and mirrored by mouse models suffering from similar biometal deregulations.

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Introduction

The brain tightly regulates the transport and metabolism of metal ions to maintain its normal physiological function. Disruption of this regulation may have detrimental effects on brain functioning. It is thus not surprising that alterations in

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biometals have been reported coinciding with neurodegenerative and neuropsychological disorders. However, it is still not well understood whether the dysregulation of biometals functions as a cause of a disorder, acts as trigger in combination with predisposing genetic factors or occurs as a side effect of a particular disease.

Many metal ions can be found in a living organism that can be differentiated into biometals and other trace metals. Trace metals are not synthesized by the body and their availability depends on the regular intake *via* our or our mother's diet (in the case of an embryo). A trace metal is considered a biometal if it plays a role in the normal functioning of the body, such as proper growth, development, and physiology of an organism. Determined by environmental and/or genetic factors, the actual level of a metal in the body may be classified as deficient, adequate, or excessive. The outcome (toxicity) of a deficiency or excessive exposure however has to consider chronic or acute occurrence. The metals calcium (Ca), copper (Cu), iron (Fe), magnesium (Mg), manganese (Mn), selenium (Se), and zinc (Zn) are examples of biometals. Besides essential biometals, there are metals found in the body that under normal conditions appear to be indifferent to the metabolism, whereas some metals are clearly toxic even at low levels. Examples include aluminium (Al), arsenic (As, a metalloid), cadmium (Cd), mercury (Hg), and lead (Pb). Especially exposure to heavy metals during pregnancy might influence brain development.[1] However, even biometals can lead to toxicity depending on their concentration. Deficiency but also the excess of one biometal may lead to toxic effects, in part by disturbing the equilibrium of other metal ions.

In addition, it has been known for decades that metal ions can be used in the treatment of neurological disorders. Lithium (Li), for example, has been proven effective in the treatment of mood disorders, especially bipolar disorder[2–4] and may have disease-modifying effects in Alzheimer's disease.[5] Moreover, zinc sulfate and acetate are key drugs in the treatment of Wilson's disease, a rare autosomal recessive disease characterized by an excess of copper in the brain and other organs.[6] The latter example also indicates that the concentration of a particular metal ion can influence the levels of other metal ions in the body. Thus, here, we will highlight alterations in biometal profiles in several exemplary neurological disorders and try to identify common motifs. Additionally, we will investigate the behavioural phenotypes of rodent models suffering from alterations in biometals or heavy metal intoxications to evaluate whether a shared pattern with disorders with similar metal ion profiles exists.

Biometals in Alzheimer's disease (AD)

The majority of cases of early-onset AD has a genetic basis (familial AD) and amyloid precursor protein (APP), presenilin 1, and presenilin 2 have been identified as mutated target genes. Intriguingly, presenilins are important for cellular Cu and Zn turnover.[7] However, most cases of AD with late onset (about 95% of all AD cases) have no clear genetic cause and a combination of genetic and environmental factors might act as risk factors for the development of AD. A number of genes such as Apolipoprotein Eε4 (APOEε4), bridging integrator 1 (BIN1), clusterin (CLU), phosphatidylinositol binding clathrin assembly protein (PICALM), and complement component (3b/4b) receptor 1 (CR1) have been identified to contribute to the likelihood to develop the disorder.[8]

Apart from these genes, various recent studies revealed that biometal dyshomeostasis plays a crucial role in the pathogenesis of AD (Table S1, ESI†). Substantial evidence indicates that disrupted neuronal homeostasis of different metal ions such as Fe, Cu, Zn and Ca may mediate synaptic dysfunction and neuronal toxicity.[9–14] According to several findings, heavy metals have been implicated in interactions with the major protein components of Alzheimer's disease namely, beta-amyloid (Aβ) and tau.

In particular, Cu and Zn are known to associate with senile plaques.[15,16] Thus, it is hypothesized that in AD, Cu and Zn sequestration into plaques leads to an abnormal distribution of these metal ions, resulting in a deficiency of Cu and Zn in the surrounding cells.[17] Furthermore, a decrease in Zn in serum and blood of AD patients, while an increase of Zn in CSF has been reported.[18–22] Additionally, Fe is associated with senile plaques[23] resulting in an increase of Fe in the neuropil of AD patients.[15,24–26] However, whether this increase alters total brain Fe levels, serum, or CSF Fe levels, or causes a local Fe deficiency in the surrounding tissue of plaques is still not well investigated.[17] Mn, similar to Cu, Fe, and Zn, shows an inverse correlation with Aβ plaque-load.[27] However, changes in trace metals in AD are complex and cannot be described as decrease or increase. The most likely reason for this is the capacity of senile plaques to sequester some metal ions that in turn become mislocalized instead of simply decreased or increased. Additionally, this mislocalization takes place in the vicinity of plaques and not throughout the whole tissue and plaques themselves show a spatially unequal distribution throughout the brain. Moreover, the distribution of metals in the brain is inhomogeneous. Therefore, findings on alterations in metal ions in AD depend very much on the tissue and specific brain region analysed. Using meta-analysis and systemic review methodologies, on bulk level, no changes in Fe, and minor changes in Zn and Cu were reported.[28] The data on Zn levels were heterogeneous and the available evidence suggested that Cu is generally depleted in AD. Fe was modestly elevated in the AD putamen over controls, but no other brain region appeared to be affected.[28]

Along with these metal ions, alterations in other metals have been described in AD. For example, lower level exposure of Hg and Pb was implicated in the etiology of AD.[29] Exposure to Pb might contribute to Aβ production,[30,31] while Hg has a very high affinity for selenoproteins[32] and leads to the displacement of the biometal Se. Serum Se levels are positively correlated with cognition in AD.[33] Moreover, although AD patients display no significant differences in serum levels of arsenic, high arsenic levels can induce hyperphosphorylation of tau[34,35] and APP transcription.[36]

The plasma levels of Al were found to be increased in AD[18] and Al was able to compete and displace biometals such as Mg,

Fe and Ca.[37] Dyshomeostasis of Ca has an increasing line of evidence to be a risk factor for AD. Ca deficiency usually occurs in adult years, the time point associated with the biggest risk of developing AD. Considering the gender differences, women are more prone to Ca deficiency and they are also more likely to get AD.[38]

Biometals in autism spectrum disorders (ASDs)

Genetic factors might be largely responsible for the occurrence of ASDs[39] that alone or in combination with specific environmental factors trigger the development of ASDs. A strong association of alterations in metal ion homeostasis and ASDs has been reported in numerous studies using serum, hair, cerebrospinal fluid (CSF) and other tissues. Especially prenatal exposure to or deficiency of specific metals may contribute to the etiology of ASDs later in life by influencing the function of ASD candidate genes.[40] The most prominent metal ion reported to be altered in ASDs is Zn.[4,40,41] Many autistic children have Zn deficiency especially in young age.[42,43] Additionally, studies using hair and nail samples of autistic patients report a significant elevation of Cu[44] and the Cu/Zn ratio was found increased in the serum of patients.[45] However, recent studies indicate that metallomic profiles of ASD patients show numerous alterations. For example, besides a deficiency for Zn, lower levels of Ca, Fe, Mg, Mn and Se as well as elevated hair concentrations for Al, As, Cd, Hg, and Pb were noted.[46–49] Other studies also report an elevation of Pb and Hg together with a significant decrease in Mg and Se in autistic subjects that was correlated with the severity of the phenotype.[44] Similar alterations were observed in urine with an increase in Al, Cd and Pb.[50] In general, these metal-alterations were highly reproducible throughout many studies and hint towards a severely affected metal ion homeostasis in ASD patients.

Although candidate genes for ASDs have been identified that are part of pathways regulating metal homeostasis such as Zinc transporter 5 (ZnT5, Solute Carrier Family 30 Member 5 (SLC30A5)), metallothioneins (MTs), metal transcription factor 1 (MTF1) and copper metabolism (Murr1) domain containing 1 (COMMD1),[2,51] mutations in these genes cannot fully account for all cases of ASD with trace metal imbalances. However, these candidate genes would argue for trace metal imbalances as causative factors rather than results of the disorder.

Reduced Zn levels in Phelan McDermid Syndrome patients, a disorder with autistic behaviour caused by the loss of the short arm of chromosome 22 and thereby the heterozygous deletion of SHANK3, a synaptic Zn binding protein, have been associated with increased incidence of seizures.[52] Seizures are a common comorbidity in ASDs. Interestingly, epilepsy was also found to be associated with alterations in biometals. Decreased levels of Zn and Cr were found in epileptic patients along with increases in Cu.[53] Intriguingly, the induction of seizures did not lead to alterations in brain metal ions in a rodent model[54] hinting towards the possibility that metal ion dysregulation might be more likely a cause than a consequence of seizures (see Table S1, ESI† for an overview).

Biometals in attention deficit and hyperactivity disorder (ADHD)

A strong genetic component was proposed in the development of ADHD and certain genes such as dopamine receptor D4 (DRD4), dopamine transporter 1 (LC6A3/DAT1), Dopamin-beta-Hydroxylase (DBH), BAI-associated protein 2 (BAIAP2), and nitric oxide synthase 1 (NOS1) have been linked to the disorder.[55] However, most likely, multiple genes jointly contribute to the development of ADHD with the involvement of environmental factors. Nos1 is known to be a Cu-dependent enzyme with Cu regulating Nos1 activity. Nitric oxide itself in turn is able to mediate the release of Zn and Cu from metallothioneins. Among the identified candidate genes, except NOS1, none is directly involved in trace metal homeostasis. However, some proteins encoded by candidate genes are influenced by trace metal concentrations such as dopamine transporters that are inhibited by Zn and DBH that show decreased activity with decreased levels of Cu and Zn.[56]

Zn deficiency was identified as a biomarker in ADHD in various studies and meta-analyses.[57] Many children with ADHD were identified to display decreased levels of Zn.[58,59] However, given that Zn regulates the dopamine transporter[60] and that evidence points to a dysregulation of the dopaminergic system as the underlying cause of ADHD, it seems possible that Zn deficiency is not only a biomarker for ADHD but contributes to its etiology. Increasing evidence also suggests that Fe deficiency might lead to ADHD symptoms *via* its impact on the metabolism of dopamine and other catecholamines.[61] Similarly, high levels of Mn are able to modify the dopaminergic system. Accumulation of Mn in dopaminergic neurons occurs *via* the presynaptic dopamine transporter[62] and increased levels of Mn have been reported in some ADHD studies. Moreover, exposure to Pb has been associated with ADHD[63] (see Table S1, ESI† for an overview).

Biometals in mood disorders (MD) and schizophrenia (SCZ)

MD and SCZ might have some overlaps, both in genetic and environmental factors and studies have shown significant evidence that bipolar disorder occurs at an increased rate in the relatives of patients with SCZ and SCZ occurs at an increased rate in the relatives of patients with bipolar disorder. Candidate genes and loci such as G72(DAOA)/G30 on chromosome 13q, Disrupted in schizophrenia 1 (DISC1) and Neuregulin 1 (NRG1) have been reported similarly for SCZ and MD.[64]

Many studies report a tight association between clinical depression and Zn deficiency.[65–69] Moreover, the severity of depressive symptoms and serum Zn levels was shown to be inversely correlated. Decreased Zn levels were also found in women with postpartum depression.[70] Additionally, decreased Ca, Fe and Se levels have been reported as risk factors for postpartum depression.[71–73] In contrast, Pb levels were found significantly higher in female depressives.[67]

Decreased levels of brain Zn have also been observed in individuals with early onset SCZ[74,75] and a study found the hair concentration of Zn and Ca to be significantly decreased while

the concentration of Cu and Cd was significantly increased in SCZ patients[76] (see Table S1, ESI† for an overview). SCZ has a very significant genetic component, however, no single gene causes the disease by itself and several genes and environmental factors have been associated with an increased risk to develop SCZ.[77] Most of the identified candidate genes can be placed in pathways dealing with glutamate signalling, dopamine and serotonin receptor signalling, GABA signalling as well as amino acid metabolism and cell signal transduction.[77] Although in some of these processes trace metals are clearly involved, no obvious connection emerges that could explain the observed biometal profiles in SCZ on a genetic basis.

Biometals in Parkinson's disease (PD)

The aggregation of α-synuclein (αS) is a hallmark of PD. αS has been shown to bind Cu and Fe and binding to metal ions enhances the αS fibrillation rate *in vitro*.[78] Analysis of biometals in PD revealed many alterations. In particular, Ag, Cd, Co, Fe, Se and Zn were found decreased in serum while an increase in Al, Ca, Cr, Hg, Mg, Mn, Pb and Cu was noticed.[79] However, other studies have found significantly lower serum Cu levels compared to controls.[80]

Multiple studies have shown a selective increase in Fe content in PD brains. Dopaminergic cells thereby appear to be especially vulnerable to damage by excessive Fe. A decrease in Neuromelanin possibly sequestering free Fe might mediate cellular toxicity[81] and free Fe may promote αS aggregation (see Table S1, ESI† for an overview).

Biometals in Amyotrophic Lateral Sclerosis (ALS)

ALS is a progressive disease that affects motor neurons leading to impaired muscle control and movement. Most cases of ALS are sporadic and not inherited in contrast to cases of familial ALS, where mutations in several genes, including C9orf72, superoxide dismutase 1 (SOD1), TAR DNA binding protein (TARDBP, TDP-43), fused in sarcoma (FUS), angiogenin (ANG), Alsin (ALS2), senataxin (SETX), and vesicle-associated membrane protein-associated protein B (VAPB) have been identified. The cause of sporadic ALS is mostly unknown and probably involves a combination of genetic and environmental factors. Variations in many genes including dynactin 1 (DCTN1), neurofilament-H (NEFH), peripherin (PRPH), and survival of motor neuron 1 (SMN1) increase the risk of developing ALS.

Mutations in the copper–zinc superoxide dismutase (SOD1) were among the first linked to familial ALS.[82] *In vitro*, the loss of Zn from SOD1 results in the remaining Cu in SOD1 to become toxic to motor neurons.[82] Furthermore, Cd is able to alter SOD1 *via* induction of metallothionein (MT) expression, which in turn may affect Zn homeostasis. In particular, Zn may be bound to increased levels of MT and as a consequence, lower availability of Zn may decrease SOD1 enzyme activity.[83] Alternatively, Cd can hamper SOD1 function by interference with its secondary structure inducing miss-folding and in part aggregation of SOD1.[83] Thus, Zn deficiency or Cd and/or Cu overload might mechanistically contribute as a risk factor to the development of ALS. Intriguingly, differences for Zn and Cu concentrations were found in serum and/or CSF of older patients with ALS.[84]

Among the other candidate genes for ALS, TDP-43 and ANG interact with metal ions. Zn, but not Cu or Fe, was shown to induce the aggregation of endogenous TDP-43.[85] ANG uses a Zn ion bound at its active site[86] and Zn and Cu increase the affinity of ANG for endothelial cell receptors and extracellular matrix components several-fold.[87]

Besides case reports linking different metals to ALS, numerous studies have demonstrated altered levels of other metals in serum, CSF, or other tissues of patients with ALS. Abnormally increased Pb levels, for example, were reported in CSF, spinal ventral horn tissue and plasma of ALS patients.[88] Elevated Pb levels seem to appear especially in later stages of the disease and seem to reflect changes associated with disease progression rather being a result of previous exposure to Pb.[88] However, in some studies the findings could not be replicated but evidence favours a model where increased Pb levels appear in ALS. Additionally, there have been reports of an association of exposure to Hg and ALS. However, although some studies and case reports hint towards increased Hg levels, no final conclusion can be made so far. Similarly, an increase in Se levels has been reported in ALS patients in few studies,[87,88] however, partially conflicting reports obfuscate the analysis of changes in metal ion homeostasis in the pathogenesis of ALS (see Table S1, ESI† for an overview).

An increase in Fe[89] and Mn[90] content of CSF from ALS patients was reported along with an increase in plasma L-ferritin, a Fe binding protein.[91] The increase in the latter has been correlated with survival time of patients.[92]

Biometals in Huntington's disease (HD)

HD is a monogenic disease caused by aberrant numbers of CAG (cytosine–adenine–guanine) triplet repeats within the Huntingtin gene. Huntingtin was found to interact with Cu,[93] but not Fe. Increased Cu and Fe have been reported in HD patients, in particular the striatum, but also in HD mouse models[93] making environmental factors appear unlikely as a cause for this deregulation. Along with increases in Cu, Cu-regulatory genes have been found induced in HD and Cu-binding and/or -chaperoning proteins proposed as a potential therapeutic strategy for HD.[94]

Alterations in brain Fe levels in turn have been associated with movement disorders in general, such as Parkinson's disease, multiple system atrophy, progressive supranuclear palsy and restless legs syndrome.[95] Additionally, accumulation of Mn occurs with an increase in Fe[96] (see Table S1, ESI† for an overview).

Biometals in prion diseases

Familial prion diseases (10–15% of all cases), such as familial Creutzfeldt–Jakob disease (CJD), Gerstmann–Sträussler–Scheinker syndrome (GSS), and fatal familial insomnia (FFI) are caused by mutations in the PRNP gene encoding for the prion protein (PrP). The other cases of prion disease that are classified as either sporadic or acquired show no mutation in the PRNP gene,

but for reasons so far not known, PrP is transformed into PrP^{Sc}, the infectious isoform of PrP.

The prion protein (PrP) is known to be a Cu binding protein.[97,98] This association with Cu may be necessary for its normal cellular function. PrP was also shown to act as a ferrireductase, its absence causing systemic Fe deficiency in a mouse model[99] which might explain Fe imbalances in sporadic CJD brains where PrP accumulates in aggregates.[99] However, other metals can bind to the PrP.[98] Among them, the displacement of Cu by Mn, might facilitate seeded polymerization.[100] Additionally, possibly due to an evolutionary link to the Zrt/Irt-like protein (ZIP) metal ion transport family, PrP might be involved in neuronal Zn homeostasis facilitating Zn transport.[101–103] Serum from mice that lack cellular PrP was found to have elevated Cu levels.[104] However, biometal profiles from patients suffering from prion diseases are not well investigated and the determination of alterations in brain or body metal ion content awaits future research (see Table S1, ESI† for an overview).

Biometals in lysosomal storage disorders (LSDs)

LSDs are a group of diseases characterized by defective lysosomal enzymes and a resulting accumulation of molecules in the lysosome.[105] CNS pathology is a common feature of LSDs. About 50 LSDs are known and many of them, such as Gaucher type 1, 2 and 3 as well as Niemann–Pick type C (NPC), are strongly linked to a dysregulation of Ca signalling in lysosomes.[106,107] However, besides Ca, imbalances in other metals have been reported. TRPML1, for example, a lysosomal ion channel implicated in the lysosomal storage disease Mucolipidosis type IV was shown to not only transport Ca but also Fe and Zn across the lysosomal membrane.[108] TRMPL1 also plays a role in regulation of Fe levels throughout the body.[109] Gaucher is also characterized by a dysregulation in Fe metabolism with chronically increased ferritin and liver Fe.[110,111]

Furthermore Cu accumulation has been associated with NPC since the NPC1 protein along with ATP7B is suspected to play a role in Cu transport and incorporation into ceruloplasmin.[112,113] However, recent results indicate a cell-type specific Cu accumulation in NPC disease.[114] Though, studies systematically investigating metal profiles in NPC and other LSDs are missing.

Biometals in other neurological disorders

There are many other neurological disorders with reported imbalances in metal ion homeostasis that we can only cover briefly in this review. Among them, Wilson's disease is an inherited disorder of Cu metabolism. This Cu overload my also influence Fe metabolism.[115] Similarly in Menke's disease, alterations in Cu levels are found.[116,117] Wilson's disease is caused by mutations in the ATP7B gene encoding for a plasma membrane Cu-transport protein. The function of ATP7B may be to incorporate Cu into apoceruloplasmin, and to release Cu. In contrast ATP7A, the gene mutated in Menke's disease is regulating the import of Cu.

Friedreich's ataxia has been reported to be accompanied by an increase and redistribution of Fe.[95,118] The mutated FXN gene encodes the protein frataxin, an Fe-binding protein responsible for forming Fe–S clusters. Some patients display low Zn values.[119] Spinocerebellar Ataxia has been shown to be associated with alterations in biometals. For example, patients with Spinocerebellar Ataxia type 2 (SCA2), caused by mutations in the ATXN2 gene, display reduced concentrations of Zn in serum and CSF[120] (see Table S1, ESI† for an overview).

Results

Part I – Characteristic biometal profiles in neurological disorders

Based on the observed alterations in metal ions described above, we tried to summarize the published alterations (Table 1).

In a next step we analyzed this data in order to identify common motifs in the pattern of metal ion dysregulations (Fig. 1, Table S2, ESI†). Our results show that for some neurological disorders, similar patterns arise. For example, the biometal profiles for Autism spectrum disorders (ASDs), Mood disorder (MD) and Parkinson's disease (PD) are only slightly different from each other.

Cu and Zn seem to have competing roles in a way that Cu overload leads to Zn deficiency and *vice versa*.[144–146] In a recent metallomics study measuring hair concentrations of 26 trace elements for 1967 children with ASD, an inverse relationship between Zn and Pb concentrations as well as Cd and Al concentrations was also observed.[147] Pb replaces Zn at binding sites of enzymes. Low Zn levels along with high Pb burden can be seen in ASD, MD, PD and ADHD.

The biometal profile of ADHD also resembles the profiles of ASD, MD and PD, however, Fe overload in ADHD is a unique characteristic. Interestingly, ASD, MD and PD share partly similar phenotypes such as depression, increased anxiety and problems in social cognition. Additionally, ADHD is often a comorbidity of ASDs.

Given that our hypothesis predicts that deficiency or overload of a particular trace metal should result in similar biometal profiles in animal models, in a next step, we thus evaluated whether these animal models display shared behavioural alterations.

Part II – Shared behavioural phenotypes associated with a particular biometal profile

Based on the results obtained above, where a certain alteration in one biometal is accompanied by simultaneous alterations in other metal ions, the question arises whether this interconnectedness of specific metals is also visible on a behavioural level. For example, if Zn deficiency is linked to Pb overload, mouse models for both, Zn deficiency and Pb overload should display similar common features in their phenotype. Therefore, we set out to investigate the literature on rodent models for alterations in metal ions (Table S3, ESI†) and documented their behavioural alterations (Table 2).

Based on the results obtained above, we conclude that for some metal ions that might be co-dependent a similar pattern

Table 1 Overview of imbalances of metal ions across several neurological diseases (* reported data are in part contradictory). PIXE: Particle Induced X-ray Emission; XFM: X-ray Fluorescence Microprobe Imaging; XRF: X-Ray Fluorescence Spectroscopy; WB: Western Blot; ICP: Inductively Coupled Plasma Mass Spectrometry; INAA: Instrumental Neutron Activation Analysis; ELISA: Enzyme Linked Immunosorbent Assay; AAS: Atomic Absorption Spectroscopy; eCp: Enzymatic Oxidase Assay; GFAAS: Graphite Furnace Atomic Absorption Spectroscopy; MRM: Magnetic Resonance Microscopy; EPR: Electron Paramagnetic Resonance Spectroscopy (= Electron Spin Resonance (ESR)); ESI-MS: Electrospray Ionisation Mass Spectrometry; MRE: Magnetic Resonance Elastography; EDX: Energy Dispersive X-ray Spectroscopy

Disease	Biometals	Method	Tissue tested	Reference
Alzheimer's disease	Cu*, Zn, Fe*, Mn↑	Micro-PIXE, XRF microprobe, XFM, WB, colorimetric, magnetometry	Senile plaques, neuropil	15, 16, 23–26, 28
	Zn↑	ICP, INAA, ELISA	CSF	21, 22, 27
	Cu, Zn↓	ICP, AAS, eCp	Serum, plasma	17–20
	Cu, Al↑			
Autism Spectrum Disorders	Ca, Fe, Mg, Mn, Se, Zn↓ Al, As, Cd, Hg, Pb↑	ICP, AAS	Hair	43, 46–49
	Cu, Hg, Pb↑ Mg, Se↓	AAS	Hair, nails	44
	Cu/Zn↑		Serum	45
	Al, Cd, Pb↑		Urine	50
Phelan McDermid Syndrome	Zn↓	AAS	Blood	52
Epilepsy	Zn, Cr↓ Cu↑		Serum	53
ADHD	Zn↓	Colorimetric, ELISA	Blood, nutrient intake	57–59
	Mn↑	GFAAS	Serum	62
	Pb↑	ICP	Blood	63
Mood disorders	Zn↓	AAS	Serum	65, 66, 68–70
	Ca, Fe, Se↓ Pb↑	AAS, colorimetric	Blood	67, 71, 73
Schizophrenia	Zn↓		Brain	74, 75
	Ca, Zn↓ Cu, Cd↑	AAS	Hair	76
Parkinson's disease	Ag, Cd, Co, Fe, Se↓ Al, Ca, Cu, Cr, Hg, Mg, Mn, Pb↑	ICP	Serum	79
	Cu↓	AAS	Serum	80
	Fe↑	WB, MRM	Brains	81
Amyotrophic Lateral Sclerosis	Cu↓, Zn↑	AAS	Serum, CSF	84
	Fe (ferritin↑, transferrin↓) Mn↑	EPR, ICP, ELISA, AAS, colorimetric, immunonephelometry	CSF, plasma	89–92, 121
	Se↑Hg, Pb, Cd↑*	AAS, NAA	CSF, spinal ventral horn tissue, blood, urine	83, 88
	Zn↓, Cu, Cd↑	ESI-MS	SOD1 proteins?	82, 122, 123
Huntington's disease	Fe, Mn↑	AAS	Brain	95, 96
Prion diseases	Mn↑ Cu↓	ICP	Blood, brain, and liverFrontal cortex tissue	98, 100, 121
Gaucher	Fe↑	MRE, colorimetric	Liver, serum	110, 111
Wilson's disease	Cu↑	EDX	Systemic	115
Menke's disease	Cu↓		Systemic	116, 117
Friedreich's ataxia	Fe↑	XRF	Brain, myocard	95, 118
	Zn↓	AAS	Hair	119
Spinocerebellar ataxia type 2	Zn↓	AAS	CSF, serum	120

of behavioural alterations occurs (Fig. 2). In particular, the results show that rodent models for adult Zn deficiency display increased anxiety and depression like behaviour. While Zn deficiency is also prevalent in patients suffering from depression,

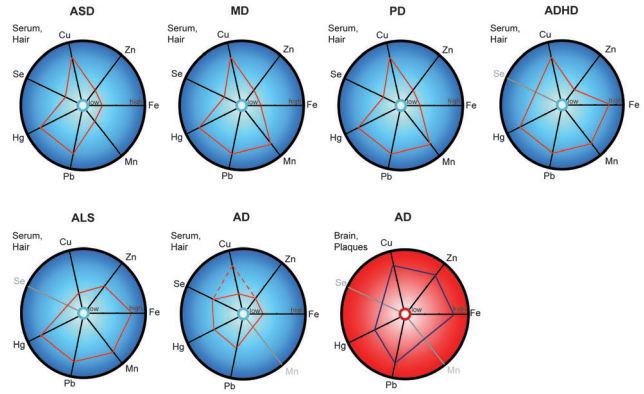

Fig. 1 Biometal profiles of neurological disorders. Using the data provided by the studies indicated above (Table 1) and additional studies,[28,80,92,121,124–143] biometal profiles for specific neurological disorders are visualized. Only studies using the same tissue samples (hair or serum) were used, except for AD where alterations in brain tissue and metal accumulation in plaques are also shown. Metal concentrations rise from the center to the outer border of the circle. ASD: Autism Spectrum Disorder; MD: Mood Disorders; PD: Parkinson's Disease; ADHD: Attention Deficit and Hyperactivity Disorder; ALS: Amyotrophic Lateral Sclerosis; AD: Alzheimer's Disease. For AD, Cu levels in serum and hair of patients are still unclear since both an increase as well as a decrease have been reported in the literature.

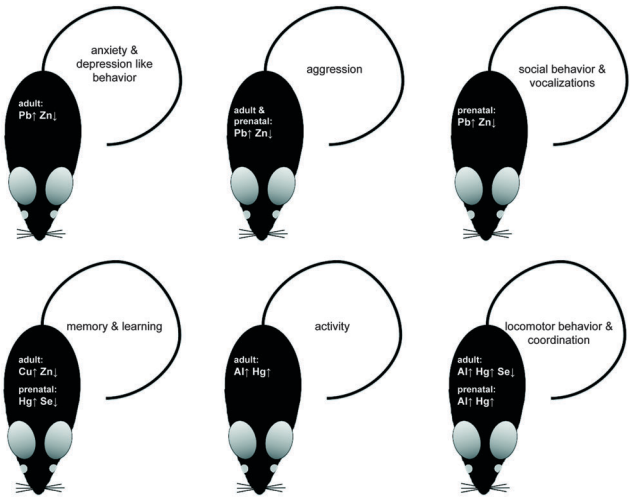

Fig. 2 Evaluation of behavioural parameters from animal models with specific metal ion deficiency or overload. Various studies report behavioural abnormalities in animal models suffering from an overload or deficiency of a specific metal ion. Based on the data shown in Table 2, similar behavioural changes can be observed for rodent models with adult Zn deficiency that display increased anxiety and depression like behaviour and rodent models for Pb overload. Additionally, aggression behaviour and social behaviour including vocalizations show significant alterations, both, in Zn deficient animals as well as animals suffering from Pb. Similarly, Cu overload as well as Zn deficiency were reported to affect learning and memory in rodent models. Hg overload and Se deficiency both cause memory and learning abnormalities as well as deficits in locomotor behaviour and coordination in animal models. Mice with Al overload similarly to mice with overload in Hg display impairments in locomotor behaviour and coordination as well as increased activity.

high Pb burdens have also been reported. Indeed, rodent models for Pb overload similarly show an increase in anxiety and depression like behaviour. Thus, one might assume that both metals act antagonistically with the overload in Pb causing a Zn deficiency and *vice versa*. This pattern is also reflected in aggression behaviour and social behaviour including vocalizations, where Zn deficient animals as well as animals suffering from Pb overload show significant alterations compared to wildtype animals (Fig. 2). Another antagonistic interaction was reported between Zn and Cu. Interestingly, Cu overload as well as Zn deficiency were reported to affect learning and memory in rodent models.

A relationship between Se deficiency and high Hg burden was reported before. Investigating the behavioural phenotypes of rodent models with Hg overload and comparing them to the phenotypes of Se deficient animals reveals that both, excess of Hg and Se deficiency cause memory and learning abnormalities as well as deficits in locomotor behaviour and coordination. Along with the increase in Hg, an increase in Al has been shown in many patients assuming a synergism between Al and Hg levels. Indeed, mouse models for Al overload similar to mice

Table 2 Overview of the effects of imbalances in metal ions on the behaviour of mice

	Metal	
Behaviour	Increase	Decrease
(A) Adult		
Locomotor behaviour and coordination		Al↑, Hg↑, Se↓, Mn↑, Cu↑
Activity	Pb↑	Al↑, Hg↑, Mn↑
Anxiety/depression like behavior	Pb↑, Mg↓, Zn↓, Cd↑	Zn↑
Memory and learning	Se↑	Al↑, Se↓, Fe↑, Zn↓, Cd↑, Cu↑
Social behaviour and vocalizations		Pb↑
Aggression	Pb↑, Mg↓, Zn↓	
Seizures	Zn↓(vesicular)	—
(B) Prenatal		
Locomotor behaviour and coordination		Al↑, Hg↑, Cu↓
Activity		Hg↑
Anxiety/depression like behaviour	Hg↑	Al↑
Memory and learning		Hg↑, Se↓, Fe↑↓
Social behaviour and vocalizations		Al↑, Pb↑, Zn↓
Aggression	Pb↑, Zn↓	
Seizures	Pb↑ (Fe↓), Zn↓(vesicular)	—

with overload in Hg display impairments in locomotor behaviour and coordination as well as increased activity (Fig. 2).

Thus, it might be possible that by manipulating one metal ion, specific biometal profiles were produced in these animal models leading to behavioural phenotypes that are associated with a particular biometal profile rather than alteration in the one trace metal. Moreover, the behavioural phenotypes, such as depression like behaviour in adult Zn deficient mice and reduced social behaviour and vocalizations in prenatal Zn deficient mice reflect symptoms of patients with depression and ASD, respectively, where similar alterations in biometals were observed.

Part III – Modelling biometal profiles in neurological diseases

Metals with similar physicochemical properties may, to some degree substitute one another. For example, if the body is subjected to Fe deficiency, the absorption of metabolically alike Mn is increased.[148] On a molecular level, various mechanisms might be responsible for the relationship and equilibrium of certain metals. For example, Zn deficiency has been reported to up-regulate the intestinal Zn-transporter (ZIP4).[149] However, ZIP4 as other transporters has an additional affinity for other metals, such as Cu and nickel (Ni) in the case of ZIP4.[150] Thus, differential regulation of metal ion transporters as adaptive response to a metal ion deficiency might increase the risk of high-uptake of other, possibly toxic metals.

Similarly, Fe and Mn are both absorbed by the transferrin (Tf) transporter and divalent metal transporter (DMT) 1 in the gastrointestinal tract and transported through the blood *via* transferrin. Absence of Fe will lead to excess Mn uptake due to elevated expression of Fe transporters.[151] Brain transport involves DMT and ZIP (ZRT, IRT-like protein) transporters, the latter is also responsible for Fe and Zn transport into the cell.[152]

Cd is able to replace Zn at metallothioneins (MTs).[153] Additionally, Cd and Al interact with Ca in multiple organ systems[153] and might lead to Ca imbalances. An increased absorption of Cd, Pb and Al might result from Fe deficiency and Pb might additionally interact with Ca.[133] A significant inverse relationship for Ca intake and blood Pb levels was reported in 3000 American children.[154]

Thus, taken together, some metals have partially overlapping properties and functions that can be evident as synergism or antagonism[155] and the outcome of a certain shift in metal ion balance might become predictable. However, the situation will become increasingly complex in case multiple metals are deficient while others occur in excess at the same time. For example, the final concentration of metal A, which is influenced by the concentration of metal B, may not be the same if the concentration of metal C is either low, adequate, or high. Such combinatorial situations are numerous in biometal profiles and impose a major obstacle for their interpretation, especially if data for only a limited number of metals are available. Thus, a software-based analysis is imperative to understand such huge combinatorial networks, where potentially every metal can act on any other metal over the range of several orders of magnitude of possible concentrations. Unfortunately, to date interaction profiles of metals and their predictability have limitations, since not all the possible interactions have been studied so far. Additionally, such interactions do not only possess a qualitative but also a quantitative component and besides facts about who is reacting with whom it will be necessary in future to also establish at what concentrations such interaction occurs.[155] Nevertheless, based on the data presented before, we set out to investigate, whether it is possible to model the observed alterations in metals using an algorithm and whether this algorithm might be used to predict currently unknown differences in biometals in neurological disorders (Fig. 3).

The objective of our approach is to use experimental data and come up with a model that can be used to predict an endpoint (phenotype, biometal profile). This prediction is achieved by ''supervised learning''. We used a model based on the concept of ''Induction'', generalizing from single observations of metal imbalances to a broader concept of relationships between trace metals. For example, we mostly relied on studies using methods such as atomic absorption spectroscopy or inductively coupled plasma mass spectrometry, reporting alterations in metals mostly in hair and serum samples from human patients for a subset of 7 metal ions. Thus, our method is designed to induce a ''model of prediction'' based on examples, partially describing the assumed underlying relationship between two trace metals. The model was implemented using SPSS and Excel.

We used rules derived from the literature presented in this review, how concentrations of certain metal ions are interrelated for our model. The selected literature listed in PubMed was chosen based on a defined search strategy that aimed to detect as much of the relevant literature as possible (Tables S1 and S2, ESI†). Study selection criteria were used to determine which studies are included in, or excluded from, the generation of relationships between trace metals. For example, inclusion criteria were (i) focus of the paper (at least two trace metal concentrations have to be determined in the same tissue sample), (ii) use of specific methodology (preferably ICP-MS and AAS studies using hair or serum samples from humans), (iii) use of specific outcome measurements (robust statistical evaluation of data). Moreover, a quality checklist was used to assess the individual studies. Only studies were included, whose results have been reproduced in multiple and the majority of all studies found, and whose results were confirmed by at least two different methodologies. Moreover, preferably, findings were reproducible across different species such as in human as well as animal studies. Although we included findings from studies using only human samples, we did not include studies using animal models whose findings were not observed in humans.

''Training sets'' (cycles) of the model consisted of a finite number of ''labels'' (metals) and ''observations'' based on partial descriptions of an underlying functional relationship between metals. The labels had continuous values with an initial value (virtual metal concentration) of $Y_{n=1} = 10$. Observations were represented in the model described by rules. Each observation is

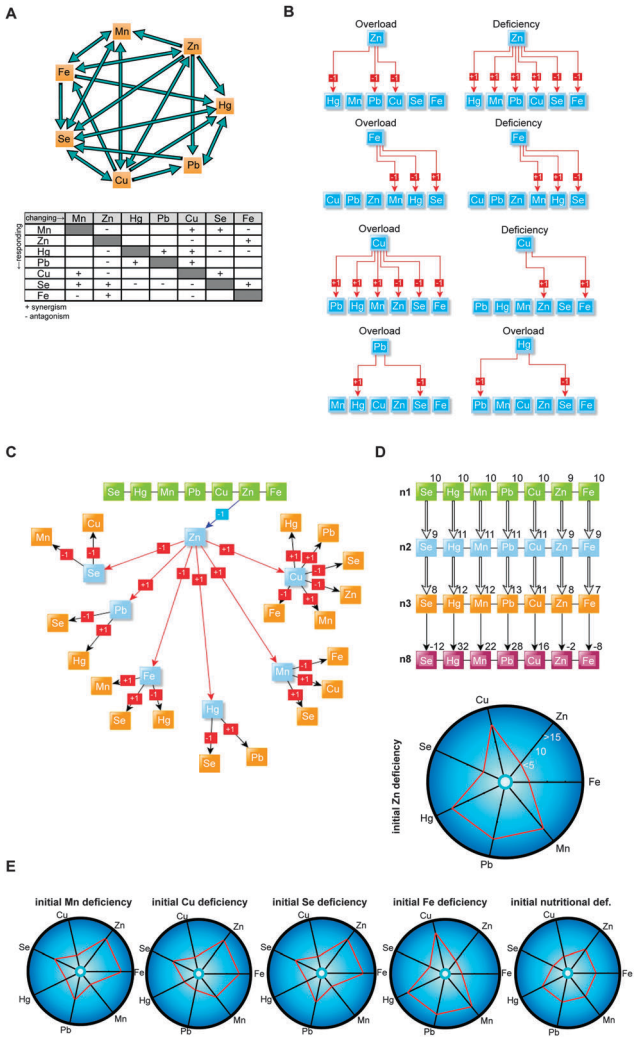

Fig. 3 Modelling biometal profiles in neurological diseases. (A) Using rules derived from literature analysis, relationships of metal ions can be classified as synergistic or antagonistic. The rules were created based on findings in hair and serum samples. As seen before (Fig. 1), the results from hair and serum measurements are quite consistent in the literature. (B) Dependent on these relationships, initial changes in the concentration of a specific metal ion will cause a chain reaction of changes affecting other trace metals. (C, D) Detailed analysis of an initial Zn deficiency. (C) Initial Zn deficiency causes a chain reaction of downstream alterations in the concentration of many trace metals. (D) The initial deficiency of Zn in the first cycle of the model (n1) affects all trace metals that are known to act in synergism or antagonism with Zn. In cycle two (n2), the virtual concentrations of these metals therefore changes as well. Since the levels of all of these metals are interrelated with the concentrations of further trace metals, further cycles (n3 to n8) again show downstream effects. The resulting metal concentrations of cycle 8 (n8) show a pattern that looks similar to the biometal profile of patients with mood disorders and Parkinson's disease. (E) The same pattern can be obtained assuming an initial Fe deficiency but not for Mn, Cu, Se, and an overall nutritional deficiency.

a set of measurements (combined relationships between two metals (rules)). Rule-based observations work in each cycle of the model on values (rules were presented as a set of IF-THEN algorithms). Thus, in each cycle, the final value for a metal and values for all metals (observation) are determined by a decision on the value of a metal that is based on the predetermined rules. These decisions can also be represented as a decision tree model that illustrates IF-THEN rules. The value Y_n is ascribed for each terminal node of a tree (final concentration of a metal). Using a decision tree, the predicted value Y is found by starting with a root of a tree (node with starting value 10 or the value from a previous cycle Y_{n-1}) and defining to which branch the observed value of a given characteristic corresponds. For example, the branch is chosen based on, whether the value for a synergistically or antagonistically related metal was decreased or increased in the previous cycle (*i.e.* considering Zn, a branch (Zn + {N}, Zn − {N}, Zn ± 0) is chosen based on: IF $Y_{\mathrm{Cu}(n)} < Y_{\mathrm{Cu}(n-1)}$, THEN $Y_{\mathrm{Zn}(n)}$ = "+1"; IF $Y_{\mathrm{Cu}(n)} > Y_{\mathrm{Cu}(n-1)}$, THEN $Y_{\mathrm{Zn}(n)}$ = "−1"; IF $Y_{\mathrm{Cu}(n)} = Y_{\mathrm{Cu}(n-1)}$, THEN $Y_{\mathrm{Zn}(n)}$ = "±0" (Fig. 4)). Considering all rules and relationships that apply for the metal, the same operations for the nodes will be repeated until a terminal node is reached. The value Y_n ascribed to the n-th node (terminal node) will be the prediction for Y_{n+1} (virtual metal concentration) in the next cycle (root).

Although decision trees allow processing of both quantitative and qualitative characteristics simultaneously, in subsequent cycles, the decision whether a synergistic or antagonistic metal was increased or decreased compared to the previous value is only based on qualitative observation without considering the actual value {N} of change.

Thus, we used rules derived from the data presented above, how concentrations of certain metal ions are interrelated to

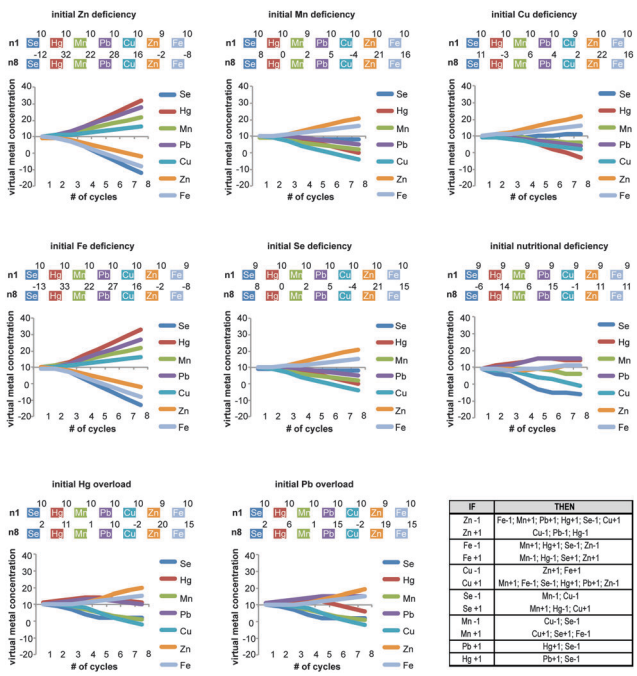

Fig. 4 Predicted alterations of virtual concentrations per metal per cycle. Based on the "if-then" rules derived from literature analysis (bottom right) the model shows an enrichment of some metals like toxic Pb and Hg occurring as a result of general nutritional deficiency. This accumulation can be reversed by the introduction of Zn in this model. Similar results can be seen modelling Pb or Hg overload where exposure to Hg and Pb in the presence of Zn limits the increase of Hg and Pb concentrations.

develop a simple model (Fig. 3A and B). For example, data show that some metal ions are synergistically regulated while the levels of others such as Zn and Cu vary in an antagonistic fashion (Fig. 3A). Therefore, the initial alteration of a specific metal ion will cause a chain reaction of changes affecting other trace metals depending on a synergistic or antagonistic regulation assuming that the initial increase or decrease is out of the buffer region of the particular metal. Examples are shown in Fig. 3B. Using simple ''IF-THEN'' relationships (Fig. 4), we set out with an initial virtual concentration of 10 for a subset of 7 metal ions chosen based on the availability of information for these metals from data of patients with neurological disorders. We then modelled the deficiency of each metal except for Hg and Pb that do not have a physiological role and should only affect the body in excess. Additionally, we investigated the case of a deficiency for all trace metals as might be seen with general malnutrition. The example for an initial Zn deficiency (Fig. 3C and D) is shown in detail. The initial deficiency of Zn in the first cycle of the model (n1) affects all trace metals that are known to act in synergism or antagonism with Zn. Thus, in cycle two (n2), the virtual concentrations of these metals will be affected, too, based on the ''if-then'' rules derived from our literature analysis. Since the levels of all of these metals are interrelated with the concentrations of further trace metals, cycle three (n3) again shows downstream effects of the initial deficiency of Zn. As an example, the resulting metal concentrations of cycle 8 (n8) are shown (Fig. 3D). Interestingly, at this point and in further cycles, the resulting pattern looks similar to the biometal profile of patients with mood disorders and Parkinson's disease and resembles the profile for ASD and ADHD (Fig. 1). We could only reproduce this pattern with an initial Fe deficiency (Fig. 3E), while Mn, Cu, Se, and an overall nutritional deficiency resulted in biometal profiles that do not reflect the profiles seen in the neurological disorders discussed above (Fig. 3E). The resulting profiles for Se and Mn deficiency are similar. These results might hint at Zn and Fe deficiency as major contributors to the alterations in biometal profiles seen in neurological diseases.

Furthermore, visualizing the alterations of virtual concentrations per metal per cycle (Fig. 4) reveals that the enrichment of some metals like toxic Pb and Hg can occur as a result of general nutritional deficiency. Here, reduction in Se is a prominent feature. However, the accumulation of Hg and Pb can be reversed by the introduction of Zn in this model and Zn seems to be the limiting factor for Pb and Hg accumulation. A similar result can be seen modelling Pb or Hg overload. In the first cycles, Hg and Pb seem to accumulate but also decrease Cu levels. A decrease in Cu leads to an increase in Zn and Fe in the model and this increase blocks a further rise in Hg and Pb levels (Fig. 4). However, exposure to Hg and Pb in presence of a Zn deficiency would increase Hg and Pb concentrations significantly.

Finally, based on this model, one might predict the concentration of some metals in patients suffering from neurological disorders, where limited information is available so far. Examples are Se in ADHD and ALS as well as Mn in serum and hair samples of AD patients (Fig. 1). Given that in ADHD and ALS, high Hg and Pb concentrations were reported along with normal to low Zn levels, and high Cu concentrations in ADHD, it is likely that a Se deficiency will be found in patients suffering from ADHD or ALS. Regarding Mn in AD, low Zn and Fe and possibly a high Cu status are predicted to lead to Mn overload and increased Mn concentration in AD patients can be expected.

Discussion

The assessment of metal ion profiles in neurological diseases shows that the biometal status is altered in almost all investigated disorders. Whether these changes are the cause, trigger in combination with a genetic predisposition, or the consequence of a disorder is sometimes not well understood (Table S4, ESI†). Additionally, given the many and diverse physiological roles these biometals play within the body and brain, it will be a major task to identify the particular mechanism by which these trace metal alterations contribute to a specific pathology. Nevertheless, from animal and human studies it is clear that the deficiency or overload of one metal ion is enough to induce a cascade of downstream alterations leading to complex behavioural changes. It is thus expected that any change in a biometal profile, no matter if cause or consequence, will modify disease symptoms and maybe progression. Thus, detailed knowledge about individual biometal profiles might open new vistas for therapeutic interventions.

However, the analysis of current data is complicated by the fact that most studies focus on one or few metal ion imbalances at a time. It is therefore hard to assess alterations in other metals. Another limitation arises due to non-homologized methods in generation of metal deficient or overloaded animal models. Various techniques have been used in the studies considered here for comparison such as supplementation *via* drinking water or supplementation/depletion *via* food pellets. Additionally, the developmental time-points and severity of metal ion alterations as well as the gender investigated vary between studies. Moreover, based on the specific research topic of the study, mostly only a subset of behaviour is investigated.

Despite these impediments, for some neurological disorders, a similar pattern in the alteration of biometal profiles can be seen. A major question therefore is, whether these changes are responsible for some shared characteristic traits between these disorders or reflect more general changes in brain activity or patient behaviour. Unfortunately, there is almost no information about the biometal profiles of animal models for brain disorders. On the other hand, analyzing rodent animal models for metal ion deficiency or overload, it seems likely that for some biometal profiles a common characteristic behaviour exists. In some cases, these behavioural phenotypes are reflected in the phenotype of human patients with a neurological disorder, where a similar biometal profile was reported. Zn deficiency for example, was not only reported in mouse models to coincide with depression-like behaviour. Multiple patient studies indicate that Zn deficiency is widespread among patients suffering from depression and panic disorders. Similarly, Zn deficiency is found in ADHD and Zn deficient animal models were reported

to display hyper-responsivity *e.g.* in situations of maternal care.[52] Interestingly, Zn supplementation therapy was able to improve symptoms in disorders associated with low Zn status such as schizophrenia[133] and depression. Zn supplementation together with Selective Serotonin Reuptake Inhibitors (SSRIs), antidepressant drugs, improves major depressive disorders more effectively compared to patients with only SSRIs.[156,157] Additionally, some studies report benefits of Zn supplementation in ADHD, although more clinical studies are needed to prove or disprove the effect of Zn in ADHD.[158] Intriguingly, using a model to obtain biometal profiles based on known interactions between trace metals, Zn deficiency alone was enough to affect other metals in a way that resembles the biometal profile of some neurological disorders. Given that Zn is the second most prevalent metal ion the brain, the demand for Zn might be high rendering the brain especially vulnerable to Zn deficiency.

Depending upon the technique used, the sensitivity of the generated data in metal concentrations varies. While quantitative methods such as atomic absorption spectroscopy (INAA), inductively coupled plasma mass spectrometry (ICP-MS) or atomic emission spectroscopy (AA), particle-induced X-ray emission (PIXE), and neutron activation analysis (INAA) have been shown to consistently produce equivalent results,[159–161] other more semi-quantitative analyses, show higher aberrations. However, here, we do not present quantitative changes but concentrate on qualitative relationships between trace metals. In future, we hope that our model is extended using more quantitative observations, where closer attention has to be paid to homologize data according to the technique used.

Given that the rules for our model are mostly based on data from hair and serum samples, the outcome of the model is also best applied to predict hair or serum levels of trace metals. Since systemic changes are considered, most likely, interferences between trace metals are modelled on the level of absorption. Interestingly, we could generate the biometal profile seen in ASD very well but were less successful in modelling others such as the biometal profile of AD. The reason might hint towards a mechanism leading to the disturbed biometal homeostasis in these disorders. Intriguingly, for some of the discussed disorders such as ASD, recent research has found a pathology of the gastrointestinal (GI) tract, sometimes referred to as ''leaky gut'' syndrome.[162–164] Similarly, a link between PD and gut inflammation has been discussed.[165] In contrast, in AD, the disturbances might have their origin on a different level than absorption in the GI tract and the rules of our model might not apply to the same extent.

The fact that behavioural changes similar to those observed in neurological disorders with similar biometal profile are seen in animal models with trace metal alterations favours a model where the imbalance of certain metals indeed is sufficient to trigger specific disease related changes. Additionally, it is likely that in some cases, metal ion dyshomeostasis might act together with a genetic susceptibility as trigger for the onset of a disorder. In other cases or additionally, metal ion dyshomeostasis might modify symptoms or disease progression. However, while there are data indicating that imbalances in metal ion homeostasis indeed contribute to the etiology of some neurological disorders, the major question why these imbalances arise in the first place remains unanswered. Given that all essential biometals have to be provided to the body by food sources, nutritional deficits might have to be considered. For example, indeed, children with ASDs show food aversions and altered eating behaviours.[166–168] However, the nutritional status of mothers during pregnancy might be more important in ASDs and maternal obesity and diabetes have been identified as risk factors for ASDs.[38] Similarly, regarding neurodegenerative disorders in the elderly population, the nutritional status of, for example, AD patients was reported to be low.[169] However, it is unlikely that eating habits are responsible for all the imbalances described in this review. Thus, other factors such as the ability to absorb trace metals should be considered. In future, further research will be necessary to determine whether the pathological process seen in some neurological disorders actually begins in the brain or elsewhere like the GI system.

Conclusions

Based on the current evidence, it is likely that imbalance in metal ion homeostasis contributes to the pathology and possibly the etiology of several neurodegenerative and neuropsychiatric disorders. Moreover, the analysis of specific patterns of dysregulation of biometals reveals that along with a deficit or overload of a certain biometal, concentrations of other metal ions are likely to be affected as well. One might even predict that although for some disorders only the alteration of a specific metal ion is reported so far, future research will reveal broader alterations of metal ion homeostasis.

Regarding a possible beneficial intervention in patients, the question therefore arises, whether all these alterations have to be treated separately. The search for specific chelators for certain metal ions and the preparation of a cocktail of chelators and metal supplements to rescue all alterations in an individualized therapy might be challenging. However, our results indicate the existence of common biometal profiles across several neurological diseases. Thus, it is conceivable that certain, if not all, biometals exist in equilibrium and the alteration of one might create downstream effects shifting the levels of other biometals in order to establish a new equilibrium. This leads to the conclusion that indeed therapeutically addressing the modification of levels of a particular biometal might resolve imbalances in general. Therefore, in future, it is necessary to understand the relationship of metal ion concentrations across different tissues and specifically within the brain parenchyma.

Taken together, here, we would like to raise awareness that research in one particular area with interest in one particular metal should nevertheless be conducted in a manner that does not neglect alterations in the big picture of metal ion homeostasis. Therefore, understanding the influence of metal ion concentrations across different disorders assessing full biometal profiles and specifically focusing on those symptoms that might be modified by a particular biometal is a highly

desired goal. Additionally, higher spatial and temporal resolution of alterations might provide novel insights into the pathomechanisms of deficiencies or overload of trace metals and might help to resolve some of the controversies as reported for example in AD.

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