

# The Emerging Role of Manganese Dysregulation in Neurological Disease

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## **Abstract**

Manganese (Mn) is an essential trace metal; dysregulation of Mn in a broad spectrum of neurological disorders is direct evidence of its vital importance in brain development and key neurophysiological processes. Historically, the observation of acquired “manganism” in drug abusers and miners provided early evidence of Mn exposure-related brain toxicity. The recent identification of inherited Mn transportopathies, causing neurodevelopmental and neurodegenerative syndromes, further corroborates the neurotoxic potential of Mn. Moreover, Mn dyshomeostasis is also implicated in Parkinson’s disease and other neurodegenerative conditions such as Alzheimer’s and Huntington’s diseases. Deciphering the common underlying Mn-associated disease mechanisms will offer greater understanding of the neurophysiological role of Mn. Current and future research will facilitate the development of better targeted personalised medicine strategies for Mn-associated neurological disorders.

## Introduction

Manganese (Mn) is an essential trace metal that is vital to all living organisms. Mn has a key role in the catalytic function of multiple metalloenzymes and forms an integrative part of many metabolic processes.<sup>1</sup> There is growing scientific interest in metal biology, given the increasingly recognised association between metal ion dysregulation and human disease. As such, dyshomeostasis of Mn is now acknowledged to be a common factor in the pathogenesis of several neurological disorders, including Parkinson's disease (PD), Alzheimer's disease (AD), Huntington's disease (HD), amyotrophic lateral sclerosis (ALS) and prion disease.<sup>2</sup> Indeed, it is not surprising that the fundamental role of Mn within the nervous system renders the brain particularly vulnerable to Mn dyshomeostasis.

As an abundant element, dietary Mn deficiency is not reported. However, emerging evidence suggests that inherited states of Mn deficiency and excess can interfere with normal nervous system function. There is growing concern that higher Mn exposure can negatively impact intellectual function, cognition, and behaviour, particularly in children's developing brains.<sup>3</sup> Mn intoxication in humans is commonly described as "Manganism", an acquired neurological condition that shares several overlapping features with PD.<sup>4</sup> The recent discovery of several monogenic disorders associated with Mn dyshomeostasis has further increased our understanding of the important sequelae of Mn dysregulation, as well as the key physiological processes that regulate metal balance.<sup>5-7</sup> Although post-mortem neuropathology and experimental research studies suggest that neurodegeneration associated with oxidative stress, aberrant protein aggregation, and neuroinflammation are the main hallmarks of Mn toxicity, the underlying mechanisms are yet to be fully elucidated.<sup>8</sup> Correlation of the pathophysiological phenotypes in commonly occurring neurological conditions such as PD, with those observed in the rarer inherited and acquired disorders of Mn dyshomeostasis could potentially reveal similarities in the molecular mechanisms underpinning these neurodegenerative diseases.<sup>9</sup>

In this review, we provide a comprehensive overview of the biological significance of Mn in disease states, with a particular focus on the neurological sequelae of Mn dysregulation. We also discuss current treatment strategies for managing disorders of Mn dyshomeostasis. Finally, the utility of different experimental models to better investigate Mn-related disorders will be discussed, bringing hope for the development of novel therapeutic approaches.

## Manganese: Brain homeostasis and neuronal function

The primary source of Mn in humans is through dietary intake. Mn levels are tightly regulated through enteral absorption, hepatic metabolism, and biliary excretion.<sup>10</sup> Erythrocytes within the vascular system mediate systemic dissemination of Mn, and metal transporters transfer Mn across cellular membranes.<sup>11</sup> The skeletal system, liver, pancreas, kidneys, and brain are reported to contain the highest levels of Mn, attributed to their high energy demand (figure 1).<sup>10</sup> The normal ranges of Mn levels in body fluids are estimated to be 4–15µg/L in blood, and 5.32–14.03ng of Mn/mg of protein in the brain.<sup>12,13</sup> In the central nervous system (CNS), Mn preferentially accumulates in the basal ganglia (especially in the globus pallidus and substantia nigra), as well as in the frontal lobe, cerebellum, and olfactory bulb.<sup>14</sup> Importantly, the half-life and elimination rate of Mn in the brain is postulated to be slower than in other organs, which may contribute to the pathological accumulation of brain Mn and subsequent neurotoxic effects in disease states.<sup>15</sup>

Brain Mn homeostasis is tightly regulated through several mechanisms at both a cellular and systems level in order to drive enzymatic processes that are fundamentally dependent on Mn. In order to bypass the blood brain barrier (BBB), Mn enters the brain by direct intra-axonal uptake via presynaptic nerve endings, through the choroid plexus, or through the olfactory epithelium and olfactory nerves.<sup>16</sup> Cellular uptake of Mn in the CNS is mediated through store-operated calcium channels (SPCA1 & 2), Mn/citrate transporters, solute carriers ZIP8 and ZIP14, and the divalent metal transporter 1 (DMT1).<sup>8</sup> Moreover, several other transmembrane transporters regulate Mn influx/efflux, including Transferrin, Ferroportin, ZnT10, DAT, and PARK9. Located on cellular and subcellular membranes, these transporters form membrane pores that control Mn transfer across cellular compartments (appendix pp 2).<sup>4,16</sup>

Mn is an essential metal for normal neuronal function and also offers neuroprotection. It is a key cofactor for astrocytic glutamine synthetase (GS), the core enzyme regulating the glutamate/Gamma aminobutyric acid (GABA)-glutamine cycle and excitatory glutamate (Glu) turnover for normal synaptic function. Whilst neuroprotective, it has the potential for neurotoxicity at high Glu concentrations.<sup>17</sup> The key metalloenzymes Arginases 1 & 2 (ARG1 & 2) and Mn Superoxide Dismutase (MnSOD) are also Mn-dependent enzymes with neuroprotective function. Whilst ARG1 & 2 catalyse the removal of abnormal protein aggregates (which could drive a neuroinflammatory response),<sup>18</sup> MnSOD protects cells against cellular stress by reducing ROS production in the mitochondria and alleviating cellular stress

that would otherwise trigger apoptosis and neurodegeneration.<sup>19</sup> Moreover, where DNA damage occurs, Mn-activated Serine/Threonine protein kinase ataxia telangiectasia mutated (ATM) and tumour suppressor protein P53 can regulate cell cycle, DNA damage repair, or trigger apoptosis.<sup>16,20</sup> Mn is also required in all tissues for protein and lipid glycosylation, as Mn is an essential cofactor for many Golgi glycosyltransferases, including the  $\beta$ -4-galactosyltransferases (B4GALTs) and UDP GalNAc:polypeptide-N-acetylgalactosaminyltransferases (GALNTs) families, responsible for normal saccharide biosynthesis in nearly all biological processes.<sup>21</sup>

## **Manganese dysregulation in neurological disease**

Both direct and indirect dysregulation of homeostatic mechanisms can cause Mn imbalance and disruption of key cellular processes, which can lead to important neurological sequelae and a number of neurological conditions (tables 1 and 2).

The developing brain is vulnerable to Mn fluctuations, as illustrated by numerous studies which suggest that exposure to higher Mn concentrations in drinking water correlate with psychological and neurological abnormalities in children, including effects on behaviour (anxiety, depression), cognition (memory deficits, lower Intelligence Quotient and academic achievement scores), and motor function (tremor, postural imbalance and coordination difficulties).<sup>22</sup>

Historically, the most striking example of acquired Mn neurotoxicity is the observation of “manganism” in drug abusers and miners, as a direct result of Mn exposure. Occupational exposure to inhaled Mn-rich fumes and dust in the mining and welding industry accounts for a large proportion of reported cases, with specific neuroimaging findings (figure 2).<sup>23,24</sup> Likewise, intravenously injected Ephedrone drug abuse can cause Mn toxicity as a result of potassium permanganate (KMnO<sub>4</sub>) required for drug synthesis.<sup>25</sup> Total parenteral nutrition (TPN), especially in neonates<sup>26</sup>, can have equally deleterious effects, as younger individuals absorb and retain greater levels of Mn compared to adults. Toxicity is further exacerbated by the nature of TPN, which bypasses the normal Mn homeostatic mechanisms in the gut and liver.<sup>26</sup> Furthermore, in Acquired Hepatocerebral Degeneration (AHD), patients with advanced hepatobiliary disease (with reduced hepatic elimination of Mn) present with excess brain manganese accumulation and associated clinical features of neurotoxicity, now recognised as a key features of AHD.<sup>27</sup>

The recent identification of three inherited Mn transportopathies with prominent neurological features provides further evidence of the importance of Mn homeostasis in the brain. In 2012, the first cases of Hypermanganesemia with Dystonia 1 (HMNDYT1) due to bi-allelic mutations in *SLC30A10*, encoding a Mn exporter (ZnT10), were reported; affected patients presented with hypermanganesemia associated with juvenile-onset dystonia, gait disturbance, bradykinesia, and truncal ataxia (OMIM #613280).<sup>6,28</sup> HMNDYT1 patients also commonly present with liver dysfunction, ranging from steatosis to fulminant hepatic cirrhosis. To date, a total of 42 cases of hypermanganesemia due to ZnT10 deficiency have been described, with a majority of early-onset but also a few adult-onset cases (age of onset 1 – 57 years).<sup>6,28-35</sup> In 2016, bi-allelic loss-of-function mutations in ZIP14 (a Mn importer encoded by *SLC39A14*) were reported in 9 patients with childhood-onset hypermanganesemia (age of onset 0.5 – 18 years) associated with early-onset dystonia, spasticity, limb contractures, and parkinsonian features (OMIM #617013, HMNDYT2) (panel 1).<sup>5</sup> Since the original report, a further 8 HMNDYT2 cases have been described, all sharing similar phenotypic features to the original cohort.<sup>36-40</sup> Both HMNDYT1 and 2 share a number of overlapping disease features: the neurological phenotype is similar, brain MRI shows a characteristic pattern of Mn accumulation (figure 2), and affected patients have elevated serum Mn levels. In contrast, bi-allelic mutations of *SLC39A8* encoding the ZIP8 transporter (Mn importer) have been identified as a new inherited cause of hypomanganesemia (OMIM #616721, Congenital Disorder of Glycosylation, type II; CDG2N). Affected patients manifest in infancy with a dramatic reduction of Mn serum levels and a severe clinical syndrome characterised by cranial asymmetry, dwarfism, seizures, and developmental delay with characteristic MRI features (figure 2); to date, 12 affected individuals have been described.<sup>7,41,42</sup>

Taken together, these monogenic disorders provide direct evidence of a link between pathogenic variants in Mn transporters, brain Mn dyshomeostasis, and neurological disease.

Given that individuals suffering from manganism present with parkinsonism, it is plausible that a degree of Mn dysregulation may also contribute to the striatonigral neurodegeneration observed in both sporadic late-onset and inherited forms of PD. Recent research has highlighted the loss of neuroprotective effects against Mn toxicity in familial PD.<sup>43</sup> Indeed, for PARK1, PARK2, PARK6, PARK7, PARK8, and PARK9-related disease, there is evidence of increased sensitivity to Mn toxicity, impacting cellular detoxification and inducing neurodegenerative cascades such as activation of lysosomal and mitochondrial stress response pathways.<sup>44-46</sup> Chronic Mn exposure similarly involves the same response pathways in sporadic and inherited

autosomal dominant (*APP*, *PSENI*, and *PSEN2*) forms of AD, further amplified by the formation of amyloid-beta plaques upon exposure.<sup>47</sup>

It is thus likely that Mn dyshomeostasis leads to loss of a neuroprotective environment in certain neurological disorders. Huntingtin protein (HTT) has been postulated to regulate metal homeostasis in the brain and its toxic gain of function in HD has been in part attributed to a Mn transport deficiency, which has been shown to affect autophagy and urea cycle metabolism in HD cellular and animal models.<sup>48-50</sup> Furthermore, it is proposed through a multiple-hit hypothesis that early exposure to Mn, associated with specific underlying genetic factors and ageing, is associated with ALS susceptibility.<sup>51</sup> Finally, Mn exposure has been shown to trigger prion protein (PrP) misfolding and accumulation, where metal binding sites of PrP render them susceptible to environmental changes; such Mn-related protein conformation may be important in the pathophysiology of prion disease.<sup>8</sup>

## **Pathophysiological consequences of Mn dysregulation**

The underlying mechanisms of Mn neurotoxicity are multifactorial, with dysregulation of a variety of cellular processes. Mn dyshomeostasis has downstream effects on a number of key functions, including dysregulation of dopamine neurotransmission, Glutamate/GABA-Glutamine signalling, induction of oxidative stress, mitochondrial defects, and lysosomal dysfunction (figure 3).

### **Neurotoxic effects of excess Mn**

Mn accumulation in the brain leads to a neurotoxic microenvironment which impacts normal neuronal physiology. There is strong evidence suggesting that Mn influences normal neurotransmitter synthesis, release, and reuptake, especially in the basal ganglia. Abnormal presynaptic dopaminergic signalling and decreased dopamine (DA) release have been observed with Mn exposure.<sup>52</sup> Mn also mediates the oxidation of DA into free radicals and DA quinones (DAQ), further promoting neurotoxicity.<sup>53</sup> Interestingly, a retrospective study by Guilarte et al, examining PET, SPECT, and MRI imaging in patients with manganism does not suggest there is loss of nigrostriatal neurons, as observed in PD.<sup>54</sup> It is therefore possible that Mn neurotoxicity may contribute to dysregulation of DA neurotransmission, without initially affecting the cellular integrity of the DA neurons in the early stages of the disease.<sup>54</sup> However, more recently, larger cross-sectional studies in Mn-exposed welders, using PET 6-[<sup>18</sup>F]fluoro-L-DOPA (F-DOPA) and D2R imaging suggest a pattern of dopaminergic dysfunction, which could indicate neuronal dysfunction or degeneration of the nigrostriatal pathway.<sup>55,56</sup> Further

longitudinal SPECT and PET neuroimaging studies on larger patient cohorts will better address this area of controversy. Mn exposure is also thought to disrupt neurotransmission in excitatory Glu and inhibitory GABAergic neurons, mainly by altering the expression of key transporter proteins (figure 3) involved in Glu and GABA turnover.<sup>57</sup> It appears that a certain Mn exposure threshold needs to be exceeded (around 0.1mg/m<sup>3</sup> respiratory airborne Mn concentration) in order to increase GABA levels and affect fine motor performance, as suggested by Magnetic Resonance Spectroscopy (MRS) studies in Mn-exposed welders, PD and hemochromatosis patients.<sup>58,59</sup>

Given the structural and functional complexity of the basal ganglia – integrating a variety of inhibitory and excitatory input and output pathways – it is not surprising that disturbances of these finely tuned processes manifest as major motor and cognitive dysfunction.

Mn is a metal with high reduction potential, which in physiological conditions has the ability to remove harmful superoxides and hydrogen peroxide. Indeed, as a cofactor to MnSOD, Mn is involved in the mitochondrial oxidative stress response.<sup>60</sup> While physiological levels of Mn are crucial for normal cellular function, excess Mn is postulated to exacerbate oxidative stress by activating oxidative stress-related protein p38 MAPK and JNK pathways.<sup>61</sup> Studies in N27 rat-derived mesencephalic dopaminergic neuronal cells have also shown that high levels of Mn activate pro-apoptotic processes such as mitochondrial cytochrome c release, caspase-3 activation, protein kinase C delta (PKC $\delta$ ) activation, and DNA fragmentation.<sup>8,62</sup> Moreover, Mn preferentially accumulates in the mitochondria and high levels of mitochondrial Mn can bind to specific substrates such as succinate and malate, interfering with mitochondrial respiration.<sup>8</sup> Excess Mn can therefore directly impair oxidative phosphorylation, inducing ROS production. Of note, the duration and levels of Mn exposure seem to influence Mn-induced mitochondrial dysfunction; studies in neuronal models have defined a threshold above which Mn exposure leads to mitochondrial dysfunction, with increased H<sub>2</sub>O<sub>2</sub> production, decreased oxygen consumption and extracellular acidification rates.<sup>63-65</sup> Mn exposure can ultimately result in mitochondrial degeneration and cell death, which further contributes to neurodegeneration. Mn neurotoxicity is also mediated by glial cells through the neuroinflammatory response. Indeed, in response to raised Mn levels, microglia release proinflammatory cytokines IL-6, TNF $\alpha$ , and IL-1 $\beta$ <sup>66</sup> and astrocytes activate p38 and NF- $\kappa$ B, which further exacerbate neuroinflammation.<sup>67</sup> Overall, Mn-induced glial cell activation can lead to production of ROS and generation of Nitric Oxide (NO). Moreover, inflammatory cytokines further propagate the inflammatory response in surrounding tissue. The presence of



such microglial cell populations within the basal ganglia may therefore contribute to Mn toxicity-induced neuroinflammation.<sup>68</sup>

A hallmark of many neurodegenerative disorders (such as PD, AD, HD, ALS, or spongiform encephalopathies) is misfolding and aggregation of specific proteins that contribute to the formation of a neurotoxic cellular and extracellular environment. In states of excess Mn, there may be disruption of cellular trafficking pathways, with impairment of the proteasome system, autophagy, and endosomal trafficking, which may then lead to abnormal protein accumulation.<sup>69</sup> For instance, excess Mn is thought to induce the exosomal secretion and oligomerisation of  $\alpha$ -synuclein, a hallmark of PD, further accentuating abnormal aggregation in this neuroinflammatory environment.<sup>44,70</sup>

### **Effects of Mn deficiency in the brain**

The ubiquitous nature of Mn in the human diet renders dietary insufficiency unlikely. However, the discovery of genetic variants in *SLC39A8* has provided insight into the pathophysiological consequences of Mn deficiency in humans. The pleotropic nature of *SLC39A8* variants is clearly exemplified by their association with a broad range of complex human phenotypes with multiorgan presentation, including cardiovascular and metabolic diseases, schizophrenia, PD, skeletal defects, epilepsy, and developmental delay.<sup>7,41,42,71-73</sup> Abnormal glycosylation profiles are universally reported; such congenital disorders of glycosylation (CDGs) are attributed to impaired activity of Mn-dependent glycosyltransferases.<sup>71</sup> For example, a type II CDG profile is evident in recessive *SLC39A8*-related disease, consistent with dysregulation of the Mn-dependent glycosylation enzyme  $\beta$ -1,4-galactosyltransferase.<sup>7,74</sup> In recessive disease associated with biallelic loss-of-function mutations, ZIP8 deficiency has been shown to induce cellular stress through mitochondrial dysfunction similar to that seen in Leigh syndrome.<sup>75</sup> Mutant protein also prevents accumulation of intracellular Mn with consequent impairment of MnSOD activity and downregulation of specific mitochondrial genes.<sup>76</sup> Overall, mitochondrial function is affected with impaired mitochondrial respiration, abnormal mitochondrial membrane potential, decreased ATP production, and reduction in redox activity. Mitochondrial dysfunction leads to increased ROS production, triggering the neurodegeneration cascade.<sup>76</sup>

### **Therapeutic approaches for disorders of Mn dysregulation**

As with other complex neurological syndromes, patients with disorders of Mn dysregulation require a tailored, holistic, and multidisciplinary approach for management of symptoms. Close

working between medical, surgical, and therapy teams will ensure optimum symptomatic treatment to promote motor and cognitive function, prevent disease-associated complications, reduce mortality risk, and improve quality of life. In the future, better screening tools and more robust biomarkers will be needed to aid diagnosis and monitor the severity of Mn-related disorders.

For hypermanganesemia disorders, chelation therapy with disodium edetate, aiming to reduce Mn levels, have been trialled in patients with variable effect.<sup>5,31,34,39,77</sup> As such, it remains a controversial therapy for hypermanganesaemia. While some patients show improvement of motor function, others have less favourable outcomes; some do not respond to treatment at all, develop side effects to therapy or continue to deteriorate over time. Some patients with monogenic, inherited causes of hypermanganesemia have been reported to experience clinical improvement (reduced Mn levels, stabilisation of gait, reduced bradykinesia, and improved dystonia) when treated with chelation therapy.<sup>5,28,31</sup> In contrast, others with genetic hypermanganesemia continue to deteriorate despite treatment and may also experience severe side effects.<sup>5</sup> Clinical response to chelation therapy is thus variable in patients with inherited forms of hypermanganesemia and may depend on factors such as patient age, clinical phenotype, disease severity, and age at treatment onset (panel 1). For acquired forms of manganism, chelation therapy generally does not always improve symptoms, and can also potentially lead to significant side-effects, as reviewed by Kwakye et al.<sup>78</sup> Clinical benefit is only rarely reported.<sup>77,79</sup> Chelation therapy presents with additional burden for patients due to the need for regular intravenous injections. Close monitoring for the potential adverse effects of chelation as well as monitoring of Ca, Zn, Cu, and Se levels are also required to avoid adversely reducing these trace metals.

Symptomatic treatment with levodopa has also been trialled in some patients presenting with hypermanganesemia, with variable clinical benefit. Indeed, clinical benefit of levodopa treatment has been reported in a number of AHD patients.<sup>80</sup> In other studies, Mn-exposed welders do not respond to levodopa, regardless of the dose, as reviewed by Marreilha Dos Santos and colleagues.<sup>81</sup> In patients with inherited Mn transportopathies, response to levodopa has shown variable clinical advantage<sup>5,28,37</sup> The lack of recent publications on this subject requires future longitudinal studies on larger cohort of patients with different Mn toxicity aetiologies, which will certainly give more insight into the efficacy and optimal dosage of levodopa in Mn-related disorders.

Mn administration is likely to be an effective therapy for patients with inherited Mn-deficiency. In a recently described study, two patients with SLC39A8 deficiency were treated with high doses of MnSO<sub>4</sub> (15–20 mg/kg/day) and both patients experienced major clinical improvement in their motor abilities and neurological symptoms.<sup>82</sup> Close monitoring of Mn is key to ensure that levels stay within the therapeutic range, to avoid excessive Mn exposure.

Despite advances in chelation therapies and symptomatic treatments, the paucity of truly disease-modifying or curative treatments renders many patients with disorders of Mn dysregulation with significant disability and increased risk of mortality; development of novel precision therapies therefore constitutes a research priority.

## Experimental models for therapeutic innovation

### Model systems and novel technologies to study Mn transportopathies

To date, disease modelling for inherited Mn transportopathies has been undertaken in a wide variety of *in vitro*<sup>5,6,83,84</sup> and *in vivo* models.<sup>85-88</sup> These studies have provided important insight into the effects of mutant protein in cell and animal model systems. Whilst acknowledging their merit, current disease models also harbour limitations as many only partially recapitulate core disease features, are limited in their representation of the human brain, and are not ideal for assessing drug efficacy. The study of human diseases associated with Mn dyshomeostasis also presents major hurdles, given the relative inaccessibility of the human brain and technical difficulties associated with *ex vivo* neuronal cultures.

Genetically engineered larger animal models, such as canine, ovine, porcine and non-human primates are likely to be a superior alternative to conventional cellular and animal models for studying Mn-related disorders; the higher complexity of their neuronal system aligns more closely with the human brain, facilitating the study of more complex neurodegenerative disorders. The sporadic occurrence of such conditions in higher order species also constitutes a potential advantage.<sup>89</sup>

Induced pluripotent stem cell (iPSC) technology also provides a powerful tool to study the pathological mechanisms of neurological diseases. Patient-derived iPSCs can be differentiated into neurons, providing appropriate human cell types to study disease and develop drug screening platforms and therapeutic approaches for clinical application. iPSC neuronal models have already been used for screening therapeutic compounds and also for cell replacement therapy for the treatment of several neurological disorders, including PD, AD, and ALS.<sup>90-92</sup>

IPSC-derived neurons share similar developmental maturation profiles to fetal neuronal cells, which renders them particularly useful for studying diseases, like some of the Mn-related disorders, where clinical manifestations first occur in infancy or prenatally.<sup>93</sup> Moreover, recent advances in IPSC-based technologies have provided three-dimensional (3D) regional-specific brain organoids, which resemble human brain structure, with several neuronal subtypes, and early post-natal characteristics.<sup>94</sup> 3D brain organoids will facilitate analysis of pathophysiological cellular phenotypes in a system that conceivably aligns more closely to patients with Mn transportopathies. IPSC-based models are also amenable to molecular stressors for aging, which may allow better recapitulation of phenotypes that appear later in disease progression.

To date, several IPSC-derived neuronal models have been employed to study Mn-associated disorders, such as HD and PD, and provided significant molecular and cellular insights on disease mechanisms.<sup>20,49,95-97</sup> However, there is currently a lack of patient-derived IPSC models for inherited Mn transportopathies and this has hampered our understanding of cellular disease progression in these rare genetic forms of manganism. It is likely that such cellular model systems, in tandem with existing *in vitro* and *in vivo* models, will certainly increase understanding of acquired and inherited forms of Mn transportopathies and facilitate the development of targeted precision therapy approaches (appendix pp 3).

Transcriptomic (using RNA sequencing) and metabolomic analysis (using high resolution metabolomics; HRM) adapted to Mn transportopathies could also reveal significant mechanisms of Mn toxicity and secondary adaptation mechanisms. For instance, studies in neuroblastoma cell models have revealed differential mitochondrial and ER-Golgi mediated gene responses as well as adjustments in energy, amino acids, and fatty acid metabolism in response to physiological and toxicological Mn concentrations.<sup>98,99</sup> These studies are crucial to determine the physiological and toxic role of Mn in a neuronal model to help discriminate between adaptive and toxic molecular responses, which could form a basis to better understand, detect, and manage adverse environmental or occupational sub-toxic and supra-toxic Mn exposure.

## Development of novel therapeutic approaches

As experimental research advances, the priority is increasingly to find better precision treatments and cures.

Current development of novel therapies is largely based on testing in transgenic animals and immortalised cell lines. Humanised models, through the use of iPSC-derived neuronal systems, may help circumvent some of the hurdles encountered by traditional approaches. Indeed, potential compounds could be assessed in iPSC-based high-throughput drug screening platforms, which have already proven to be effective for other disorders.<sup>100</sup> Moreover, these models could also reveal patient-specific, mutation-dependent drug-responses, forming the basis of an individualised, personalised medicine approach.

Transcriptomic analysis, and especially the recent rise of single cell RNA sequencing (scRNAseq), have provided insights into CNS cellular complexity, unravelling potential disease-causing pathways associated with neurological disorders.<sup>101</sup> Focusing scRNAseq on both inherited rare disorders as well as more common, sporadic diseases associated with Mn dysregulation could potentially uncover common pathological molecular signatures, thereby highlighting shared molecular targets for therapeutic innovation. scRNAseq is now evolving as a robust technology with wider application, such as detection of microRNA.<sup>102</sup> MicroRNA (miRNA) studies could facilitate development of new therapeutic strategies, by regulation of disease-altered biological pathways.<sup>103</sup> Given that the dopaminergic system is particularly affected by Mn toxicity, recent studies have evaluated the expression profile of miRNAs in dopaminergic models. RNA sequencing analysis of SH-SY5Y cells treated with Mn have revealed differential expression of 73 miRNAs. The study highlighted that specific Mn transporters, such as PARK9, ZIP8, ZIP14, or ZnT10, are particularly targeted by miRNA upon Mn exposure; specifically has-miR-4306 was shown to target *PARK9*, by reducing its expression following Mn exposure.<sup>104</sup> A recent PD study also demonstrated that Mn exposure causes overexpression of SNCA and FGF-20 by reducing the levels of miR-433 and miR-7, highlighting the potential link between manganese exposure, altered miRNA expression, and PD.<sup>105</sup> These studies have therefore highlighted the importance of miRNA in regulating pathways affected by Mn-induced neurotoxicity, and hold great promise for the future development of targeted therapeutic approaches. Indeed, it may be possible to partially or fully restore the cellular sequelae of Mn toxicity by either blocking or inducing specific miRNA expression.<sup>106</sup> Overall, RNA-based therapeutics (including antisense oligonucleotides, mRNA,

miRNA, aptamers, and small interfering RNAs) is a rapidly evolving field and will certainly facilitate the development and acceleration of therapeutic strategies tailored to patients with Mn-related disorders.<sup>107</sup>

Gene therapy, particularly the use of adeno-associated viral (AAV) vectors to deliver gene replacement for diseases associated with loss-of-function is already a therapeutic reality for some inherited neurological disorders.<sup>108</sup> Targeted delivery of AAV vectors to specific areas of the CNS, such as intraparenchymal delivery to the substantia nigra pars compacta (SNpc) in AADC deficiency (NCT02852213) and intraputaminial delivery in PD (NCT01973543, NCT03065192, NCT02418598, NCT01621581) are the subject of ongoing clinical trials. These studies provide clear precedent for future translation of AAV-based gene therapy for the inherited Mn transportopathies. Furthermore, the delivery of gene therapy using CRISPR-Cas9 technology has already been shown to restore phenotypes in laboratory models of PD<sup>109</sup>, AD<sup>110</sup>, ALS<sup>111</sup>, and HD.<sup>112</sup> Gene therapy is no doubt challenging, and success will depend on refinement of technical modalities and delivery systems. Nevertheless, the therapeutic potential of gene therapy for inherited Mendelian Mn transportopathies offers exciting future possibilities.

## **Conclusion and future directions**

The study of Mn-related disorders has led to greater understanding of the aetiological basis and pathological consequences of Mn dysregulation in the brain; indeed, such research has highlighted the critical role of finely balanced Mn homeostasis in normal brain function. Despite diagnostic advances, there is currently a striking paucity of treatment strategies for Mn-related diseases, which at present rely mainly on palliative care and symptom control. The clinical need for better refined precision treatments is now driving experimental research to develop new models to study these diseases. Patient-derived iPSC neuronal systems and larger transgenic animal models of Mn dysregulation will no doubt further improve our current understanding of Mn in health and disease states, at a molecular, cellular, and systems level. Furthermore, better laboratory models, together with more comprehensive cross-sectional studies and novel biomarker discovery will certainly help tailor the experimental landscape for the discovery of new therapies, forming the basis of future personalised medicine approaches.

## **Search strategy and selection criteria**

References were mainly identified by searches of PubMed between 1<sup>st</sup> of January 2015 and May 2021, written in English. A few additional references from key articles published before 2015 were also included. Combinations of the following search terms were used: “slc39a14”, “slc39a8”, “slc30a10”, “manganese”, “dystonia”, “parkinsonism”, “hypomanganesemia”, “hypermanganesemia”, “manganese neurotoxicity”, “transportopathies”, “neurological diseases”, “Huntington’s disease”, “ALS”, “Parkinson’s disease”, “prion disease”, “Alzheimer’s disease”, “manganese exposed workers”, “welders/smelters” were used. The final reference list was generated on the basis of relevance to the topics covered in this Review.

## **Authors’ contribution**

D.B., supervised by M.A.K., carried out the literature review, D.B. wrote the first draft of the manuscript, A.K.S.S. collated neuroimaging data, D.B, S.B., A.K.S.S., and M.A.K. revised the draft and contributed to the final version of the manuscript.

## **Conflicts of Interest Statement**

The authors declared no conflicts of interest.

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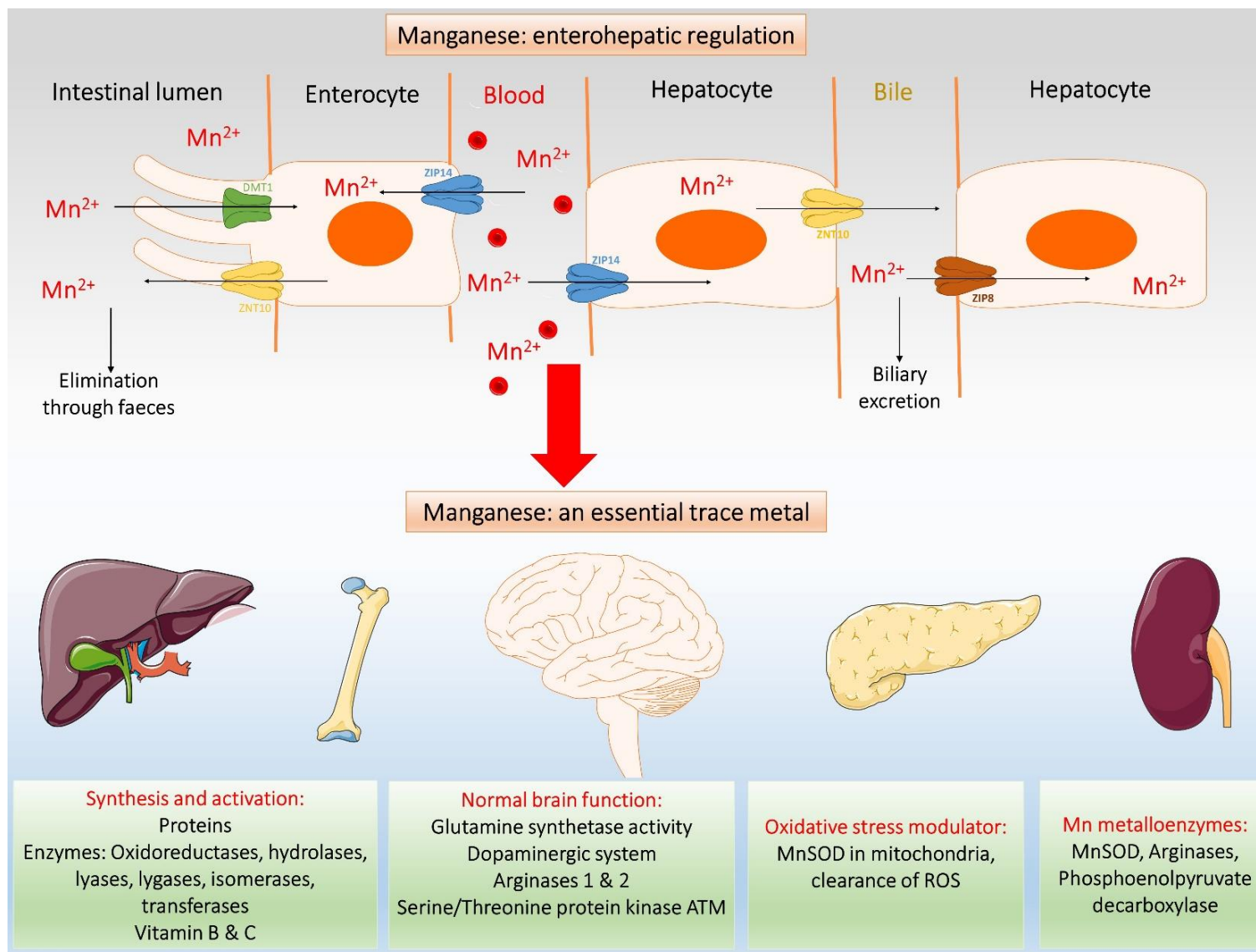
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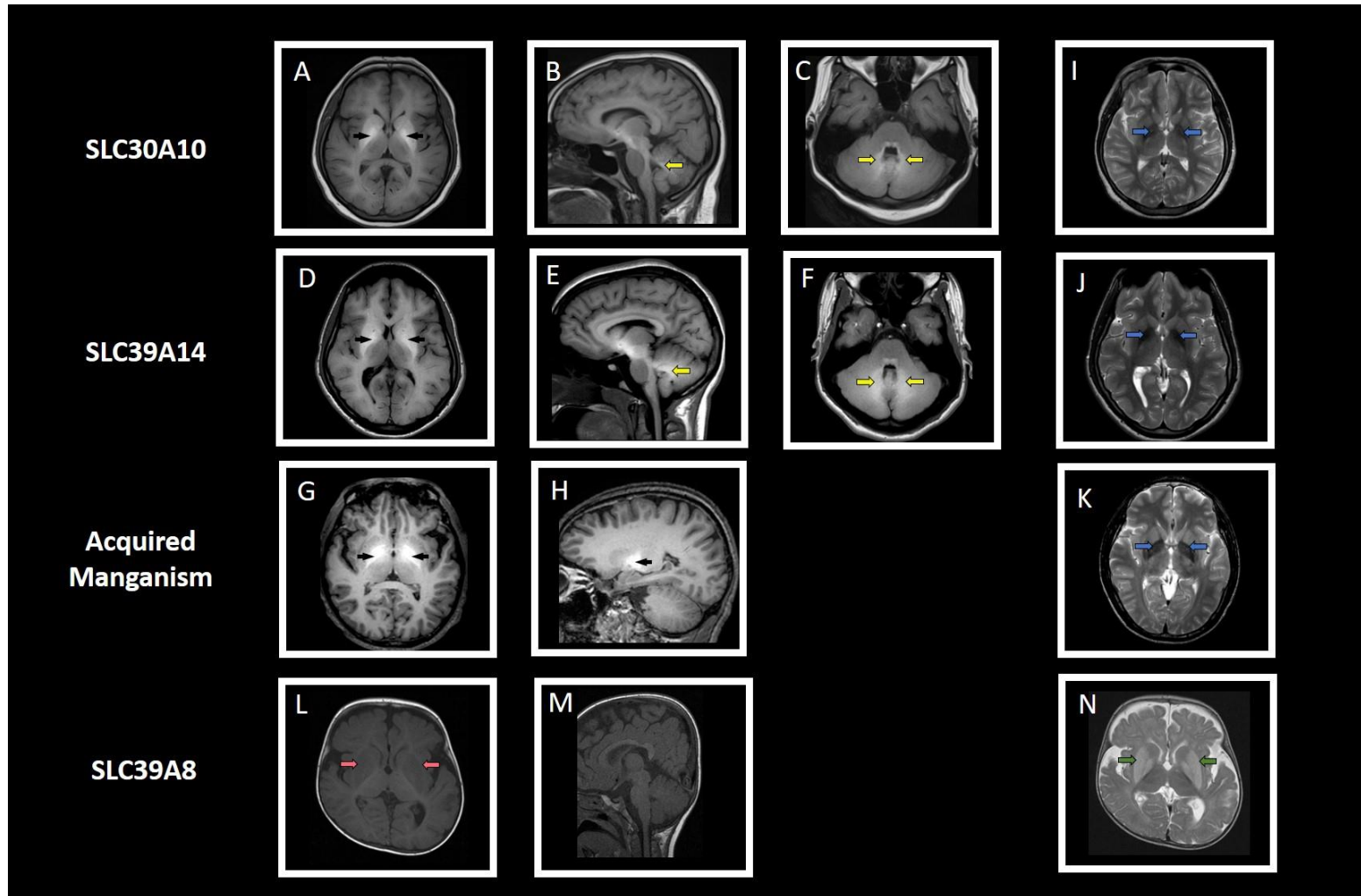
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## Figures and tables



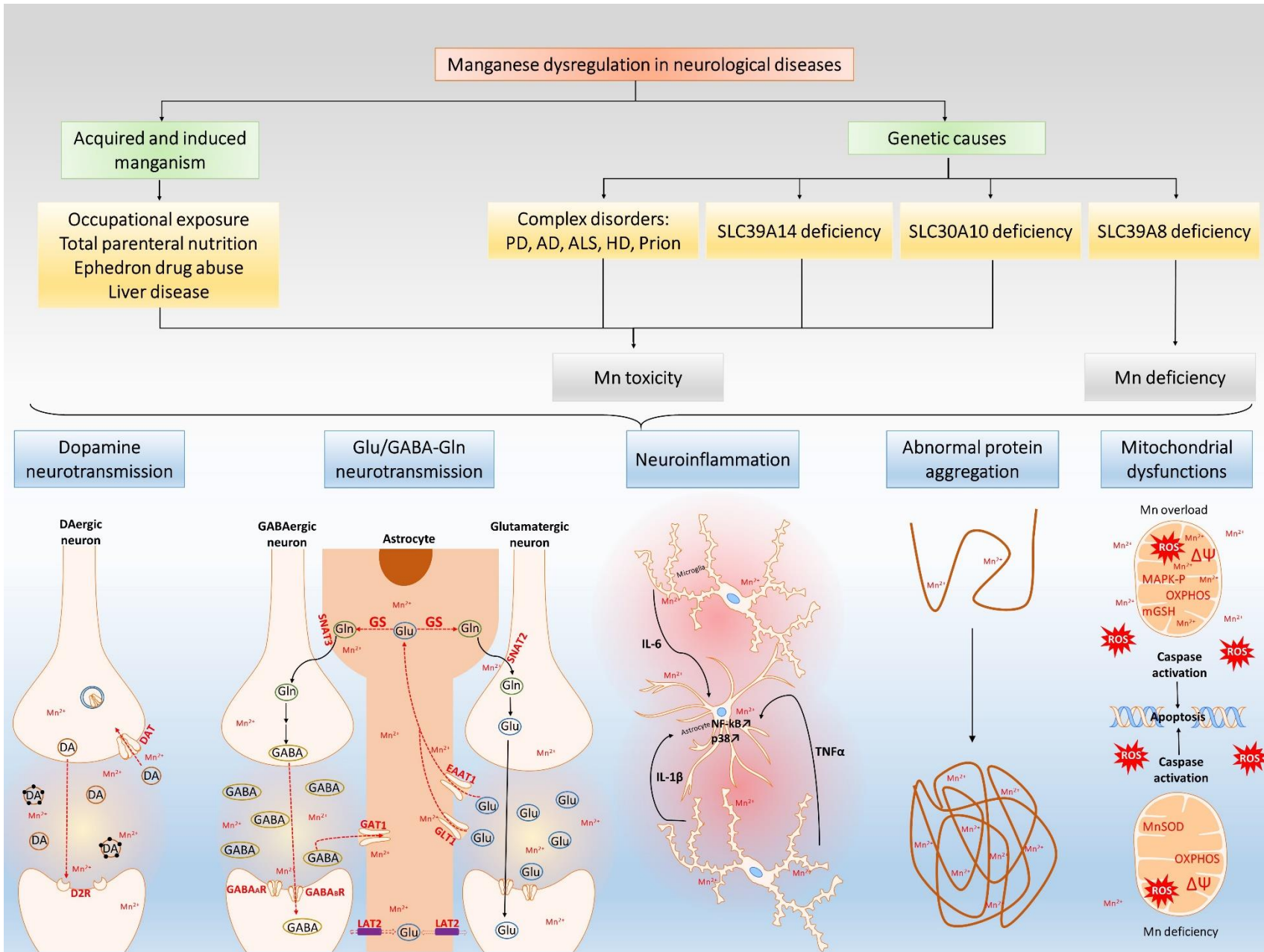
**Figure 1 – Manganese is an essential trace metal with key physiological functions.** Dietary Mn enters the enterocyte from the intestinal lumen via DMT1, and then enters the bloodstream for systemic distribution. The liver, skeletal system, brain, pancreas, and kidneys are particularly Mn-rich organs. Mn is a key constituent of certain metalloenzymes. Also, it is required for the function of numerous enzymes, as well as the synthesis of proteins and vitamins. In addition, Mn is a modulator of oxidative stress release and its function is crucial for normal brain activity. The homeostatic regulation of Mn is regulated through intestinal absorption and biliary excretion, with the help of key Mn transporters ZIP14, ZIP8, and ZnT10. ATM=ataxia telangiectasia mutated. DMT1=divalent metal transporter. Mn=manganese. MnSOD=manganese superoxide dismutase. ROS=reactive oxygen species. ZIP=Zrt- and Irt-like Protein.



**Figure 2 – Neuroimaging features in genetic and acquired manganese disorders.** Brain MRI scans of those with *SLC30A10*, *SLC39A14*, *SLC39A8* and acquired manganism (secondary to occupational exposure) are shown here. The paramagnetic nature of manganese results in distinct patterns on brain MRI scans when it accumulates in sufficient quantities in the brain. Manganese accumulation in the brain results in hyperintensity seen on axial and sagittal T1-weighted MRI images (A-H) particularly in the basal ganglia

(black arrows) and cerebellum (yellow arrows), including the dentate nucleus (yellow arrows in boxes C and F). The basal ganglia appears hypointense (blue arrows) on T2-weighted MRI images (I-K). In *SLC39A8* there is hypomanganesemia manifesting as T1-weighted hypointensity (orange arrow in box L) and T2-weighted hyperintensity (green arrow in box N) of the basal ganglia. MRI=magnetic resonance imaging. *SLC30A10*=solute carrier family 30 member 10. *SLC39A14*=solute carrier family 39 member 14. *SLC39A8*=solute carrier family 39 member 8.





**Figure 3 – Pathological consequences of Mn dysregulation in the brain.** Regardless of the cause, Mn dysregulation in humans commonly results in neurological impairments. Mn exposure can affect normal neurotransmission of DA by decreasing DA release, by reducing the expression of DA specific transporters (DAT) and receptors (D2R), and by oxidizing DA (black dots). Mn can also affect normal neurotransmission of Glu and GABA, by affecting the activity of the GS, by reducing the expression of specific transporters (SNAT2/3, GAT1, GLT1, EAAT1, LAT2) and receptors (GABA<sub>A/B</sub>). Activation of microglial cells is also induced following Mn exposure, with production of proinflammatory cytokines (IL-6, TNF $\alpha$ , IL-1 $\beta$ ) and activation of p38 MAPK and NF- $\kappa$ B by astrocytes, creating an inflammatory microenvironment. Due to impairment of the proteasome system, autophagy, and endosomal trafficking upon Mn exposure, abnormal protein aggregation is one of the hallmark of Mn neurotoxicity. Finally, Mn imbalance can induce mitochondrial dysfunction, inducing the production of ROS which can cause caspase activation and mitochondrial degeneration. In red: proteins and pathways affected by Mn dyshomeostasis and subsequent downstream effects. AD=Alzheimer's disease. ALS=Amyotrophic Lateral Sclerosis. DA=dopamine. DAT=dopamine transporter. D2R=dopamine receptor D<sub>2</sub>. EAAT1=excitatory amino acid transporter 1. GABA=gamma-aminobutyric acid. GABA<sub>A</sub>R=GABA<sub>A</sub> receptor. GABA<sub>B</sub>R=GABA<sub>B</sub> receptor. GAT1=GABA transporter 1. Gln=glutamine. GLT1=glutamate transporter 1. Glu=glutamate. GS=glutamine synthetase. HD=Huntington's disease. IL-6=interleukin 6. IL-1 $\beta$ =interleukin 1 beta. LAT2=L-type amino acid transporter 2. MAPK-P=mitogen-activated protein kinase phosphorylated. mGSH= mitochondrial glutathione. NF- $\kappa$ B= nuclear factor kappa-light-chain-enhancer of activated B cells. Mn=manganese. MnSOD=manganese superoxide dismutase. OXPHOS=oxidative phosphorylation. PD=Parkinson's disease. p38=p38 mitogen-activated protein kinases. ROS=reactive oxygen species. SLC30A10=solute carrier family 30 member 10. SLC39A8=solute carrier family 39 member 8. SLC39A14=solute carrier family 39 member 14. SNTA2 & 3=system N amino acid transporter 2 &3. TNF- $\alpha$ =tumor necrosis factor alpha.  $\Delta\Psi$ =mitochondrial membrane potential.

**Table 1 – Salient clinical features of acquired manganism.**

	Occupational	Substance Abuse	Excessive intake	Impaired hepatic elimination
<b>Route of exposure</b>	Inhalation	Intravenous	Oral or intravenous	Systemic circulation accumulation in advanced liver disease such as Acquired Hepatocerebral Degeneration (AHD)
<b>Risk factors</b>	Miners, smelters, steel manufacturing workers or welders, dry-cell battery factory workers	Ephedrone abuse (due to potassium permanganate used for illicit production), Meperidine abuse (due to incorrect synthesis to MPTP)	Food, infant milk and water with high levels of manganese; total parenteral nutrition	Portosystemic shunting and/or iron deficiency anaemia in advanced hepatobiliary disease or liver cirrhosis
<b>Motor symptoms</b>	Symmetric parkinsonism Bradykinesia Rigidity Dystonia Resting tremor Plantar flexion – “cock walk” (especially if very high exposure) Postural instability	High steppage gait Balance difficulties Dysarthric, low-volume speech Hypomimia Parkinsonism (bradykinesia, rigidity)	Gait disturbance Dysarthric, low-volume speech Developmental delay Dystonic posturing Seizures Parkinsonism	Symmetric parkinsonism Early gait disturbances or falls Prominent postural or action tremor Minimal resting tremor Dysarthria: slow, monotonous and slurred speech Cranial dyskinesia: protrusion and retraction of tongue and lips with grimacing face Unlikely to have ballism
<b>Cognitive and neuropsychiatric manifestations</b>	Paranoid psychosis Emotional lability Irritability Hallucinations	Emotional lability	Memory deficits Lower IQ and academic achievement scores Anxiety Depression	Cognition generally preserved and mild impairment if affected. Neuropsychiatric: disinhibition, aggression, apathy, paranoia
<b>Neuroimaging</b>	T1-weighted hyperintensity of the basal ganglia on MRI High pallidal index (signal in GP divided by frontal WM multiply 100) Raised GABA levels in thalamus and basal ganglia on MRS	T1-weighted hyperintensity of the GP, striatum and midbrain on MRI Normal SPECT	T1-weighted hyperintensity of the basal ganglia (especially the GP)	T1-weighted hyperintensity of the GP, SN and putamen on MRI T2-weighted hyperintensity of the cerebellum and middle cerebellar peduncles may be present
<b>Biomarker</b>	Raised manganese level in blood* Raised manganese in toenails	Raised manganese level in blood*	Raised manganese levels in blood +/- urine*	Raised manganese levels in blood and CSF*
<b>Treatment management</b>	Remove source of exposure Variable response to levodopa Manganese chelation and para-aminosalicylic acid have been tried but without significant clinical improvement	Abstinence from illicit drug use Not responsive to levodopa	Remove source of exposure Monitor trace elements whilst on total parental nutrition	Liver transplant for liver failure (may reverse neurological features) Variable response to levodopa
<b>Histological findings</b>	Intact DA neurons of the SNpc Neuronal loss in the GP, striatum and SNr Absence of Lewy bodies	NA	NA	Neuronal cell loss and spongy degeneration of deep cortical areas of frontal, parietal and occipital lobes Laminar and pseudolaminar necrosis in cortex Neuronal loss and atrophy throughout deep nuclei and cerebellum

\*Blood levels of manganese may be raised but its level does not always correlate well with the degree of neurotoxicity or clinical manifestations. Also, due to liver activity, whole blood Mn may be lowered relatively quickly, and therefore may not represent an ideal biomarker for diagnosing or monitoring acquired manganism.<sup>113</sup>  
CSF = cerebrospinal fluid. DA = dopaminergic. GABA = gamma-aminobutyric acid. GP = globus pallidus. IQ = intelligence quotient. MPTP = 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. MRI = magnetic resonance imaging. MRS = magnetic resonance spectroscopy. SNpc = Substantia nigra pars compacta. SNr = substantia nigra pars reticulata. SPECT = single photon emission computed tomography. WM = white matter.

**Table 2 – Salient clinical features of inherited manganese transportopathies and other neurodegenerative disorders associated with Mn dysregulation**

	<b>Hypermanagesemia with dystonia 1 (OMIM 613280)</b>	<b>Hypermanagesemia with dystonia 2 (OMIM 617013)</b>	<b>Hypomanagesemia disorder or congenital disorder of glycosylation type IIa (OMIM 616721)</b>	<b>Parkinson’s Disease</b>	<b>Huntington’s Disease</b>	<b>Alzheimer’s Disease</b>	<b>Amyotrophic Lateral Sclerosis</b>
<b>Causative gene(s)</b>	SLC30A10	SLC39A14	SLC39A8	PARK1, 2, 6, 7, 8, 9	HTT	APP, PSEN1, PSEN2	SOD1, C9orf72, FUS, TARDBP
<b>Age of onset</b>	Childhood and adult-onset Usually before 10 years of age	Childhood, usually before the age of 3	Birth, first year of life	Idiopathic: >65y Early-onset: <50y Juvenile: <21y	Late onset: 4 <sup>th</sup> to 5 <sup>th</sup> decade of life	Early onset (rare): 3 <sup>rd</sup> to 4 <sup>th</sup> decade of life Late onset (common): >65y	Early onset: 3 <sup>rd</sup> to 4 <sup>th</sup> decade of life Late onset: >60y
<b>Risk factors</b>	N/A	N/A	N/A	Repeated head trauma Heavy metal toxicity Pesticide toxicity Obesity Substance abuse Farming	Environmental factors such as Mn and other metals toxicity can contribute to age of onset	Repeated head injury Heavy metal toxicity Lifestyle	Repeated head trauma Smoking Environmental factors such as heavy metal toxicity Lifestyle
<b>Motor symptoms</b>	Gait disturbance Dystonia “Cock walk” gait Truncal ataxia Bradykinesia	Resting tremor Bradykinesia Dystonia Spasticity Limb contractures Loss of ambulation	Hypotonia Dystonia Limb stiffness Spasms and seizures	Resting tremor Bradykinesia Dystonia Rigidity Postural instability Asymmetrical and progressive with time	Involuntary movements Chorea Dystonia Akinesia	Motor function usually preserved but can develop motor signs such as parkinsonism	Muscle weakness and atrophy Spasticity Loss of ambulation
<b>Cognitive status/neuropsychiatric manifestations</b>	Mild cognitive impairment	Mild cognitive impairment	Cognitive impairment Intellectual disabilities	Cognitive disturbance Emotional liability Hallucinations Dementia	Behavioural changes Neuropsychiatric disturbance Dementia	Memory loss Language deficits Irritability Dementia	Can lead to frontotemporal dementia and behavioural impairments
<b>Neuroimaging</b>	T1-weighted hyperintensity of the basal ganglia and white matter T2-weighted hypointensity of the basal ganglia PET scan normal	T1-weighted hyperintensity of the basal ganglia, white matter, & cerebellum	T1-weighted hypointensity of the basal ganglia T2-weighted hyperintensity of the basal ganglia Cerebellar atrophy PET scan normal	T1-weighted MRI imaging unremarkable, PET scan abnormal (reduced uptake of 18-fluoropoda), VBM normal	Volume loss in striatum	Atrophic changes visible in later stages of disease	Abnormal T2-weighted MRI of the white matter

		T2-weighted hypointensity of the basal ganglia PET scan normal					
<b>Mn blood levels</b>	High to very high	High to very high	Low to undetectable	Normal to mildly increased	Normal	Normal to slightly decreased	Normal
<b>Treatment management</b>	Poor response to Levodopa Chelation therapy with disodium calcium edetate, with variable effectiveness	Poor response to Levodopa Chelation therapy with disodium calcium edetate, with variable effectiveness	Mn supplementation Galactose supplementation General good response to treatment	Good response to Levodopa Dopamine agonists MAO-B inhibitors Deep brain stimulation	Responsive to Levodopa Symptomatic management	Symptomatic management	Symptomatic management
<b>Histological findings</b>	DA neurons of the SNpc intact Neuronal loss & myelin loss in basal ganglia Liver cirrhosis in some cases	Neuronal loss in globus pallidus & cerebellum No apparent $\alpha$ -synuclein accumulation	Neuronal loss in cerebellum	Loss of DA neurons in the SNpc Globus pallidus & striatum intact $\alpha$ -synuclein accumulation in Lewy Bodies	Neuronal loss in the striatum, cortex, thalamus, cerebellum	Neuronal loss in the cerebral cortex, amyloid plaque formation, neurofibrillary tangles	Loss of upper and lower motor neurons (motor cortex and brainstem), inclusion bodies
<b>Molecular hallmarks</b>	Oxidative stress Mitochondrial dysfunction Neuroinflammation DA synthesis normal	Oxidative stress Mitochondrial dysfunction Neuroinflammation DA synthesis normal	Oxidative stress Mitochondrial dysfunction (Leigh-like) Congenital disorder of glycosylation type II DA synthesis normal	Oxidative stress Glutamate excitotoxicity Mitochondrial dysfunction Neuroinflammation DA synthesis reduced	Proteasome dysfunction Induction of autophagy Mitochondrial failure Apoptosis	Oxidative stress Apoptosis Mitochondrial dysfunction Abnormal protein aggregation Neuroinflammation	Oxidative stress Mitochondrial dysfunction Excitotoxicity Abnormal protein aggregation

DA=dopamine. MAO-B=monoamine oxidase B. MRI=magnetic resonance imaging. PET=positron emission tomography. SNpc=substantia nigra pars compacta. VBM=voxel-based morphometry.

## Panel 1 – Case study of a classical case of SLC39A14 deficiency

### Clinical summary

A 3-year-old girl of Indian descent, born to consanguineous parents presented with increasing ‘clumsiness’ when writing and drawing and recent onset of tip-toe walking. She had been born at term, via an elective Caesarean section and had an uneventful neonatal period. Prior to her presentation, she had normal early development, having achieved her developmental milestones at the appropriate times. There was no known history of excess environmental exposure to manganese. Gradually over time, she lost the ability to stand and walk independently but her cognitive skills remain intact. Examination at 5 years of age revealed marked hypomimia, tremor and dyskinesia in the upper limbs and dystonia in the lower limbs. On examination, she had generalised hypertonicity and hyperreflexia, with ankle clonus, tendoachilles contractures, and extensor plantar reflexes. There were no cerebellar signs.

### Key Investigations

- *Whole-blood manganese level (normal range 73– 325nmol/L) – 963 nmol/L – raised*
- *Blood lactate (normal range 0.3–1.3mmol/L) – 1mmol/L – normal*
- *Nerve conduction studies – normal*
- *Visual evoked potentials – normal*
- *Brain magnetic resonance imaging – Hyperintensity on T1-weighted sequence (with corresponding hypointensity on T2-weighted sequence images) in the deep grey matter structures particularly of the globus pallidus but also of caudate and putamen. There was also T1 hyperintensity of the pituitary gland. Generalised white matter hyperintensity was also present on T1-weighted images including the cerebellum, spinal cord, dorsal pons with sparing of the ventral pons. No cerebellar atrophy.*

### Diagnosis

- Screening of *SLC30A10* negative
- Sanger sequencing of *SLC39A14* – Homozygous protein truncating variant: c.477\_478del, p.S160Cfs\*5

### Response to Treatment

She was initially tried on baclofen and tetrabenazine with modest clinical benefit as well as levodopa which did not lead to significant improvement of her symptoms. Following diagnosis at 5 years of age, she was commenced on monthly cycles of intravenous manganese chelation therapy (500mg twice daily Na<sub>2</sub>CaEDTA for five days) for five years and then subsequently has been on 6-week cycles of chelation infusions. There was biochemical improvement of her urinary manganese level (0.39micromol/L, normal range <0.85) and her blood counts, liver function tests, electrolytes, iron studies and trace element levels have remained normal whilst on chelation therapy. The chelation treatment led to cessation of upper limb tremor and dyskinesia as well as improvement of lower limb dystonia. She also gained the ability to walk independently, wearing ankle foot orthosis.

### Clinical course

Currently aged 12 years, she continues on the 6-weekly cycles of intravenous manganese chelation infusions along with oral baclofen, tetrabenazine, zinc, ferrous fumarate and folic acid. She recently underwent surgery (tendon transfer and releases) for lower limb contractures. Her condition has remained static with no further deterioration. She walks independently with the help of a walker and foot orthoses, and has normal fine motor, vision, hearing, speech and social skills. She has not had a formal assessment of cognition but remains in mainstream education. On examination, she has normal facial expression, no bradykinesia, no upper limb tremor, dyskinesia or tremor. Lower limb spasticity, rigidity, weakness (3/5 on MRC power scale) and dystonia have persisted.