Homocysteinemia and Its Neurological Effects

5

The neurological effects of homocysteinemia start in utero. Though the exact mechanism of neural tube defects in folate deficiency is not known, it is believed that the increase in homocysteine and consequent decrease in methyl donors (e.g. *S*-adenosyl methionine) probably contribute to these defects.

Decreased SAM results in hypomethylation which causes epigenetic reprogramming and overexpression of several genes. This conforms to the fact that many genes have been implicated in NTDs, with each gene defect being associated with a specific subtype of NTD (Bhargava et al. 2014). Also, adverse fetal outcomes are known to be associated with placental pathology. Prothrombotic states, including homocysteinemia, may result in placental vascular insufficiency and a compromised fetus (Haj Mouhamed et al. 2011).

Even though it is known that folate deficiency increases homocysteine, the reverse may also be true; homocysteinemia may interfere with the absorption and action of folate by interfering with the regulation of expression of the genes for folate receptor α and reduced folate carrier I (two of the four specific folate receptor/transport proteins) (Farkas et al. 2013; Steinfield et al. 2009). Thus, homocysteinemia may be at the helm of many known disorders due to folate deficiency.

Folate deficiency is known to cause neural tube defects, and it is proposed to act through the ensuant homocysteinemia. It has been shown by several scientists that periconceptual folate supplements prevent neural tube defects.

At the same time, Steen et al. (1998) have elucidated that cobalamin has an equal role in neural tube defects, being significantly low in the amniotic fluid of fetuses with NTD. Zhang et al. (2009) found that low levels of folate and B_{12} in maternal serum increased the risk of neural tube defects. It has further been demonstrated that folate supplement does not correct a deficient state; rather it corrects the metabolism of homocysteine towards the formation of methionine. Thus, it is the methionine synthase-based reaction (which is promoted by folate as well as B_{12}) which has been shown to be abnormal in women who have pregnancies with neural tube defects

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(Mills et al. 1995). Thus, supplementing with periconceptual folate as well as B_{12} is equally important for the neurological outcome of the pregnancy.

5.1 Neurotoxicity

It is known that homocysteinemia exerts its neurotoxic effects through several mechanisms. These effects and their mechanisms could be summarized as in Fig. 5.1. These have been amply demonstrated in mice models of homocysteinemia.

Rhodehouse et al. used the $CBS^{+/-}$ mice as models of homocysteinemia and C57BL/6 as controls. They demonstrated an increased permeability of the blood–brain barrier (BBB) in the mice with homocysteinemia. Using the Morris water maze, they also demonstrated cognitive impairment in these mice (Rhodehouse et al. 2013). This was found to be mediated through NMDA (*N*-methyl D-aspartate) receptor-dependent regulation of adherens (VEC/β-catenin) and tight junctions (claudin-5) (Beard et al. 2011).

One of the mechanisms, as shown in Fig. 4.1, is through activation of MMP 9 by competitive binding of homocysteine to the postsynaptic GABA-A receptors, thus antagonizing this receptor and leading to activation of MMP-9, while at the same

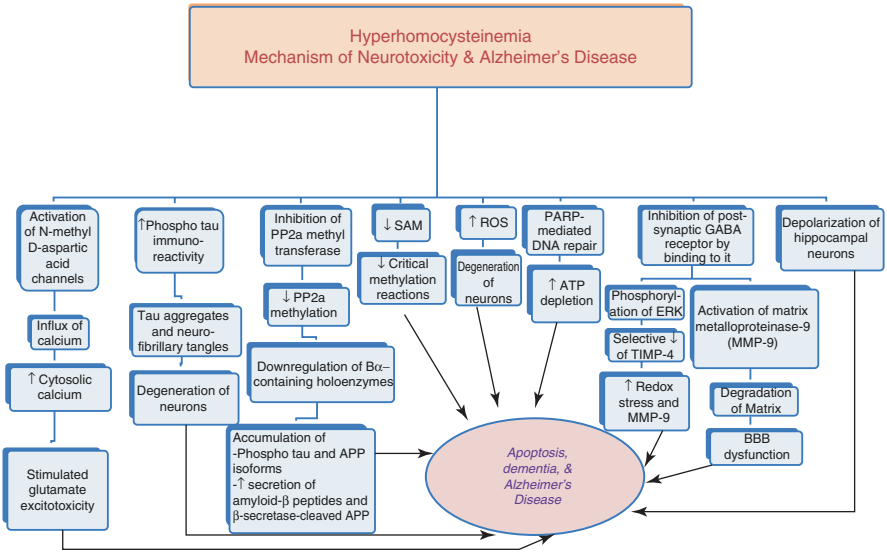

Fig. 5.1 Neurotoxicity of homocysteinemia. The neurotoxic effects of homocysteinemia are numerous impacting several molecules and mechanisms, as shown in this figure. The sequelae are, therefore, varied, ranging from neural tube defects to epilepsy to apoptosis and dementia. Through several mechanisms, homocysteinemia affects the neural tissue (including its vasculature) to result in varied pathologies through neurodegeneration and apoptosis. Homocysteinemia can result in cognitive decline and finally Alzheimer's disease through three major mechanisms—increased MMP-9, increased tau aggregates and degradation of hippocampal neurons (Modified from: Bhargava S, Bhandari A, Choudhury S. Role of homocysteine in cognitive impairment and Alzheimer's disease. IJCB 2017; DOI https://doi.org/10.1007/s12291-017-0646-5)

time, there is decreased activity of MMP-2 and TIMP-4. This has been very eloquently demonstrated by Lominadze et al. in their experiment on MMP 9 knockout mice (Lominadze et al. 2012).

In view of the experiments confirming the role of MMP-9 in homocysteinemia-induced cognitive dysfunction, we compared the auditory cognitive abilities (new object recognition test (NORT)) of $CBS^{+/-}MMP\text{-}9^{-/-}$ ($CBS^{+/-}$ mice in whom the MMP-9 gene was ablated) with those of $CBS^{+/-}$, $MMP\text{-}9^{-/-}$ and wild-type (WT) mice. It was observed that indices of cognitive abilities significantly improved ($p = 0.006$ for discrimination index and $p = 0.003$ for recognition index as compared to $CBS^{+/-}$) by ablation of the MMP-9 gene (Bhargava et al. 2014).

5.2 Cognitive Impairment, Dementia and Alzheimer's Disease

The processes of memory, learning, reasoning, attention, problem solving, decision making and language are grouped together in the term 'cognition'.

"With the current increase in geriatric population due to increased longevity, cognitive decline is becoming more prevalent and there is a need to investigate the possible causes, especially modifiable ones, so as to be able to institute preventive measures (Bhargava et al. 2017)."

A report by McCaddon et al. as early as 1998 demonstrated that early onset of Alzheimer's disease was associated with high homocysteine levels as opposed to normal homocysteine in age-matched controls without Alzheimer's disease (mean Hcy of patients = 21.9 μmol/L; mean Hcy in controls 12.2 μmol/L; $p < 0.0001$).

Wang et al. (2001) did a 3-year follow-up on individuals ≥75 years of age who were not on any vitamin supplements, specifically vitamin B_{12} and folate. Serum levels of the vitamins were measured in these randomly selected 370 subjects. Three years later, it was elucidated that those with lower B_{12} (<150 pmol/L) and folate (<10 nmol/L) were at twice as much risk (RR = 2.1; 95% CI) of developing Alzheimer's disease than those with normal levels of these vitamins. In those subjects who had a good baseline cognition, this risk was even higher (RR = 3.1; 95% CI). Even when the cutoffs of B_{12} and folate were increased to 250 pmol/L and 12 nmol/L, respectively, the pattern did not change.

Hippocampal width is known to decrease with age. That there is a relation between this decrease in hippocampal width and homocysteine was demonstrated by Williams et al. (2002) when he showed that in patients of Alzheimer's disease, there was an association of homocysteine with the atrophy of the medial temporal lobe of the hippocampus.

One of the well-known features of Alzheimer's disease is the presence of amyloid plaques. Impaired DNA repair sensitizes the hippocampal neurons to amyloid toxicity. In an in vitro study, Kruman et al. (2002) showed that in the presence of methionine and folate deficiency, the consequent homocysteinemia resulted in increased DNA damage, decreased DNA repair, increased β amyloid and increased apoptosis of the hippocampal neurons.

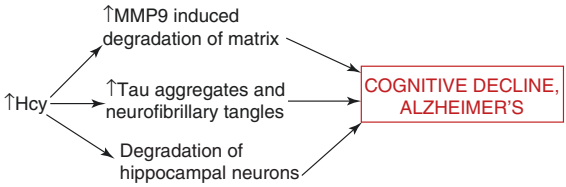

Fig. 5.2 Mechanism of homocysteinemia leading to cognitive decline and Alzheimer's disease. Homocysteinemia (**a**) induces MMP-9 expression and thereby increased degradation of the brain matrix, (**b**) enhances phosphorylation of tau protein and thereby their aggregation and formation of neurofibrillary tangles and (**c**) causes degradation of the hippocampal neurons resulting in altered functioning of the hippocampus. All these result in cognitive decline of varying degrees

It has been described that about 22% of the geriatric population has cognitive impairment. Of these 10–15% of those who have homocysteinemia progress to dementia, whereas only 1–2.5% of those without homocysteinemia proceed to dementia (Plassman et al. 2008; Edland et al. 2002; Petersen et al. 2001). As is evident in Figs. 5.1 and 5.2, occurrence of Alzheimer's disease due to homocysteinemia may be described through several of the mechanisms of neurotoxicity.

Several scientists have described homocysteinemia as an independent risk factor for cognitive impairment. Quadri et al. (2004) demonstrated that subjects with relative folate deficiency, i.e. those in the lowest folate tertile, showed a significant reduction in cognitive abilities and had an odds ratio of over three for development of mild cognitive impairment and dementia, whereas those with homocysteinemia had an odds ratio of more than four for the same.

Another study associated high homocysteine, coupled with low B vitamins, with decreased cognitive function (Tucker et al. 2005). Malouf et al. showed that asymptomatic elderly subjects with homocysteinemia when given 800 mcg of folate per day exhibited a marked improvement in global functioning ($p = 0.033$), memory storage ($p = 0.006$) and information processing speed ($p = 0.016$).

Similarly, when patients of cognitive impairment with homocysteinemia, already being treated with cholinesterase inhibitors, were given 1 mg of folate per day, they demonstrated a better overall response ($p = 0.02$) along with an improved Nurse's Observational Scale for geriatric patients ($p = 0.002$) (Malouf and Grimley 2009). In a 4-year follow-up study of 816 dementia-free subjects, Ravaglia et al. elucidated that homocysteinemia conferred a hazard ratio of 2.08 for dementia ($p = 0.002$) and 2.11 for Alzheimer's disease ($p = 0.011$) (Ravaglia et al. 2005).

Figure 5.3 delineates, in short, the mechanism involved in homocysteinemia-induced cognitive decline. Homocysteinemia, initially, inhibits the postsynaptic GABA receptor and induces increased synthesis of MMP 9. This results in the degradation of the brain matrix. Homocysteinemia also promotes the phosphorylation of the microtubule-associated tau proteins.

As these processes continue, the phosphorylated tau proteins aggregate and ultimately form neurofibrillary tangles.

At the same time, homocysteinemia increases the expression of the amyloid precursor protein, a membrane protein of the neurons, leading to an accumulation of this

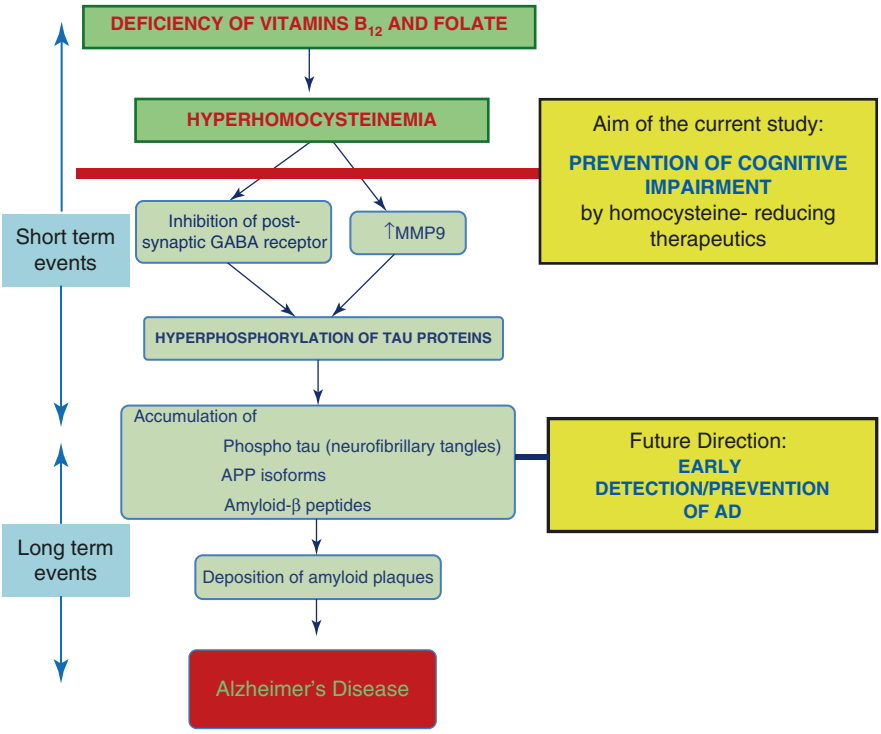

Fig. 5.3 Long-term and short-term events in the progress of vitamin (B_{12}, B_6, folate) deficiency and homocysteinemia-induced cognitive decline and its prevention. The inhibition of postsynaptic GABA receptors and the increased MMP9 production due to homocysteinemia with resultant hyperphosphorylation of tau proteins could be described as the early or preclinical events in cognitive decline; accumulation of the phosphorylated tau proteins and amyloid-β peptides into neurofibrillary tangles and amyloid plaques could be described as the successive long-term events which ultimately manifest as cognitive decline or Alzheimer's disease

protein with resultant formation of amyloid plaques which have been implicated as a cause of Alzheimer's disease. Both these processes can take several years.

But is there evidence that treating homocysteinemia with B_{12} and folate prevents further cognitive decline? McCaddon (2006) presented a case series showing a cessation of decline as well as improvement in cognitive abilities after supplementation with these vitamins. His results are summarized in Table 5.1.

Aisen et al. (2008) conducted a 2-year case-control study on 409 subjects with mild to moderate Alzheimer's disease (MMSE = 14–26) and divided them into two groups—one receiving high doses of B vitamins (5 mg folate, 25 mg B6 and 1 mg B_{12}) and the other receiving only a placebo. Only 168 subjects completed the study successfully. After 2 years of this treatment, the subjects were measured homocysteine and the cognitive subscale ADAS-cog (Alzheimer's disease cognitive scale). The results showed that the vitamin supplements significantly reduced brain atrophy probably by reducing homocysteine but did not significantly affect cognition.

Table 5.1 Effect of vitamin supplements on homocysteine and cognitive scores

Case no.	Homocysteine in µmol/L	MMSE or alternate score	Vitamin supplement with duration	Repeat MMSE (or alt score)	Repeat homocysteine in µmol/L
1	20.1	12/28	B_{12} and folate 1 month	28/30	7.5
2	27.5	Moderate to severe dementia	B_{12} and folate 3 months	Mild confusion	6.6
3	15.6	12/28 (6CIT)	B_{12} and folate 3 months	28/30	9.6
4	14.6	8/28 (6CIT) 16/39 (TICs-m)	B_{12} 6 months	21/39	8.3

6CIT six-item cognitive impairment test, *TICs-m* telephonic interview for cognitive status modified (Modified from: Bhargava et al. IJCB 2017; doi:https://doi.org/10.1007/s12291-017-0646-5)

Similarly, a meta-analysis of all double-blind, placebo-controlled randomized trials from the Cochrane Dementia and Cognitive Improvement Specialized Register Group revealed that, in healthy elderly subjects with some form of cognitive impairment, vitamin supplements with folate (with or without B_{12}) significantly reduced homocysteine but did not significantly impact cognitive parameters (Malouf and Grimley 2009).

Hence, it may be suggested that more studies are required to ascertain the effects of homocysteine on cognition and prevention of neurofibrillary tangles and amyloid plaques. Yet, homocysteinemia should be treated early to prevent its long-term effects. It may, thus, be possible to prevent, or at least delay, dementia, Alzheimer's disease or any other form of cognitive impairment. Experimental evidence of the effects of early management of homocysteinemia in terms of delay in cognitive impairment needs to be established.

5.3 Autism and Neural Tube Defects

By virtue of its definition, autism could be a subset of cognitive impairment as it includes deficits in social communication and relationships, verbal communication, language impairment and repetitive/restrictive behaviour. Tu et al. (2012), in their preliminary prospective cohort study on the role of amino acids in autism, elucidated a significantly higher plasma level of homocysteine in autistic children as compared to age- and sex-matched controls. This could be a risk factor or an association due to the poor eating habits and food selectivity of these children, resulting in multivitamin deficiencies including deficiency of B_6, B_{12} and folate which are known to increase homocysteine. Ali et al. (2011) had also reported similar findings. Kaluzna-Czaplinska et al. (2011) demonstrated significantly increased levels of homocysteine in the urine of autistic children.

Autism has also been linked to intrauterine and postnatal folate deficiency. Schmidt et al. (2012) demonstrated that in the first month of pregnancy, mothers with normal children ($n = 278$) had a significantly higher intake of folate than those

with autistic babies (n = 429). With a mean folate intake of ≥600 μg, the risk of autistic spectrum disorders was markedly reduced (adjusted OR = 0.62; p = 0.02), and with increasing folate there was a further significant reduction in risk (p = 0.001). They also demonstrated that the association between high risk of autistic spectrum disorders and low folate was highest among those mothers who had a variant methylene tetrahydrofolate reductase gene (MTHFR C677T). Frye et al. (2013) demonstrated the presence of folate receptor autoantibodies in the serum of 75% of the 93 autistic children enrolled in their multicentric study at Arkansas, New York and Melbourne. They postulated that autism spectrum disorders could be a result of cerebral folate deficiency due to the folate receptor autoantibodies. Many of these autistic children improved on leucovorin[1] therapy.

Similarly, low vitamin B_{12} levels have also been demonstrated in autistic children. Zhang et al. (2016) found serum vitamin B_{12} levels threefold lower in autistic children as compared to age- and sex-matched controls. Since deficiencies of both folate and B_{12} have been implicated in autism, it has been suggested that the mode of action of these deficiencies in causing autism is through the increased levels of homocysteine. Enough studies do not yet exist to substantiate this statement, and those that exist have included very few subjects. Also, these studies are equivocal as to the relationship between homocysteinemia and autism.

Neural tube defects (NTDs) are a failure of closure (or reopening) of the neural tube (the precursor of the central nervous system) during development of the fetus. Since the neural tube closes around the 27th day of conception and the pregnant women generally becomes aware of her pregnancy only after the third week of conception, preventing NTDs by folate intake at that time would be ineffectual. Hence, it has been suggested that periconceptual folate supplement is of prime importance (Gupta and Gupta 2004). It is yet unclear how folate deficiency causes an NTD, but it has been suggested that folate deficiency interferes with MTHFR and MS-dependent reactions, leading to homocysteinemia and decreased methyl donors. Also, folate is required for purine and pyrimidine synthesis and thereby DNA synthesis; with a deficiency of folate, therefore, neither DNA synthesis nor its methylation proceeds normally, leading to epigenetic modification of the DNA and consequent developmental defects (Feng et al. 2013).

As mentioned above, functionally diverse genes including regulators of actin dynamics, cell adhesion, electron transport and DNA repair have been implicated in the causation of NTDs, such that each gene is associated with a specific NTD. The disruption of these genes is believed to be caused by alterations in their methylation due to homocysteinemia consequent to deficiency of folate and B_{12} (Wallingford et al. 2013).

In addition to NTDs and the thrombotic effects on the placental vessels, homocysteinemia has been shown to have direct deleterious effects on the developing embryo, e.g. reduced embryo survival rate, decreased cell proliferation and decreased protein expression of the Pax 1/9 and Sox 9 genes in mesenchymal nuclei (Kobus et al. 2013).

[1] Leucovorin is a formyl derivative of tetrahydrofolate and is easily converted to the reduced folate derivatives. It, therefore, functions as the vitamin in absence or deficiency of the latter and allows for some purine/pyrimidine syntheses and, thus, DNA synthesis. Hence, it circumvents the effects of folate deficiency.

It has also been demonstrated that the development of the vascular beds is impaired in embryos exposed to homocysteinemia, unlike the controls as well as the folate-deficient embryos. There was a significant reduction in vascular area of the embryos treated with homocysteine. These vascular beds were comprised of mainly small diameter vessels; in contrast, the controls and folate-deficient embryos had mostly medium-diameter vessels. There was also a reduced expression of VEGF-A (vascular endothelial growth factor-A) and VEGFR-2 (vascular endothelial growth factor receptor-2). It is likely that this altered vasculature, along with the altered expression of the growth factor and its receptor, lead to impaired cell proliferation and a consecutive cascade of events resulting in developmental defects of the cardiovascular system (Oosterbaan et al. 2012).

5.4 Epilepsy

γ-amino butyric acid (GABA) is an inhibitory neurotransmitter in endothelial cell layer. Homocysteinemia is known to be a risk factor for neuroinflammatory and neurodegenerative diseases.

It has been demonstrated by Tyagi et al. (2007) that homocysteine competitively binds to the GABA receptor-A on the postsynaptic neuron. Homocysteinemia, therefore, causes inhibition of this inhibitory neurotransmitter. Also, it acts via the extracellular signal-related kinase (ERK) signalling pathway and thereby leads to increased redox stress and matrix metalloproteinase-9 activity and reduced activity of tissue inhibitor of metalloproteinases 4 (TIMP 4). The result is disruption of blood–brain barrier, increased microvascular permeability and increased excitatory neuronal impulses. One of the manifestations is epilepsy (Fig. 5.4).

In 2009, Tyagi et al. (2009) treated the cultured brain endothelial cells with muscimol, a GABA receptor A agonist and showed that it restores TIMP 4 activity and mitigates homocysteine-induced activation of MMP-9 as well as redox stress, confirming that in the brain, homocysteine acts through competitive inhibition of the GABA receptor A.

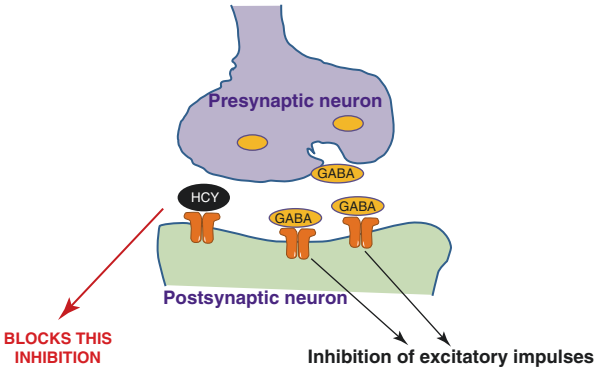

Fig. 5.4 Homocysteinemia and epilepsy. In the presence of homocysteinemia, the GABA released by the presynaptic neuron has to compete with homocysteine for attachment to the GABA receptors on the postsynaptic neurons. Hence their inhibitory effect is mitigated with resultant increased excitatory impulses and epilepsy

5.5 Parkinson's Disease

The role of homocysteinemia in other neurological disorders is controversial. In Parkinson's disease (PD), it has been established that homocysteinemia occurs in patients who are being treated with L-DOPA, the best known treatment for Parkinson's disease. This is because L-DOPA gets methylated via catechol-O-methyltransferase (COMT) which utilizes a methyl group from the remethylation cycle of homocysteine metabolism, enhancing the reaction towards homocysteine formation, thus leading to homocysteinemia (Miller et al. 1997). This may perpetrate the progression of Parkinson's due to the advent of neuropsychiatric events, cerebrovascular disease and other comorbidities as Hcy is a risk factor for neurological and vascular diseases. Also, homocysteine is an NMDA receptor agonist, which leads to neurotoxicity and dyskinesias called L-DOPA-induced dyskinesia (LID). Hence, it has been suggested that Parkinson's patients on L-DOPA should be given concomitant tolcapone, a COMT inhibitor, to prevent homocysteinemia (Müller and Kuhn 2006; Muller 2008). Kocer et al. (2016) performed an experiment to assess the effectivity of these COMT inhibitors in these patients. They observed that though homocysteine was raised in the group of patients being treated with L-DOPA and normal in those being treated with only dopamine agonists, the difference was not statistically significant. They also observed that the COMT inhibitors did not seem to prevent the development of homocysteinemia.

Zoccolella et al. (2010) published an overview of 30 studies which were involved in establishing the role of homocysteine in Parkinson's disease. They observed that the relationship between MTHFR genotype and serum concentrations of the vitamins B_{12} and folate (the major determinants of circulating homocysteine levels) was inconclusive—MTHFR genotype-related results were contradictory, and the vitamins' deficiency was associated with only an insignificantly increased incidence of Parkinson's disease. The in vitro studies showed that homocysteine resulted in a dose-dependent depletion of dopaminergic mesencephalic neurons; the in vivo brain administration of homocysteine was found to result in motor and behavioural changes similar to those seen in Parkinson's. Thus, the possibility that homocysteinemia may contribute to the causation of Parkinson's disease is still uncertain.

5.6 Multiple Sclerosis

Multiple sclerosis (MS) is considered to be an autoimmune inflammatory condition characterized by demyelination in the central nervous system with axonal degeneration and neuronal loss.

Ramsaransing et al. demonstrated that:

- Vitamins B_{12} and folate were not significantly different in controls and patients of MS.
- After correcting for the vitamin levels, there was a significant difference in homocysteine concentrations in controls and patients of MS (4.5 μmol/L higher in patients).
- Homocysteine was not significantly different in patients of MS with or without progressive disease.

Thus, homocysteinemia does not seem to have a role in pathogenesis or progression of disease in these patients. Instead, it seems to be a result of the disease process being produced in excess of homocysteine rather than having a decreased removal (as implied by the similar vitamin levels in the controls and the patients).

Astrocytes have been demonstrated to be activated during the course of the disease, and these are known to produce and secrete homocysteine. Hence, it may be possible that homocysteinemia seen in MS may be secondary to activation of astrocytes and disruption of the blood–brain barrier (Ramsaransing et al. 2006).

There are a few reports that indicate that this raised homocysteine in MS patients may lead to the increased occurrence of vascular and other neurological comorbidities.

5.7 Peripheral Neuropathy

Since homocysteine affects the nervous system through a variety of mechanisms, several scientists performed experiments to elucidate its role in peripheral neuropathy.

Bruce and Young (2008), in their community-based study on 483 adults, established through multivariate logistic regression that, after correction for age, sex, low socio-economic status, low education, HbA_{1c} and smoking, homocysteine was an independent risk factor for peripheral neuropathy. Also, it was found that it exacerbates existing peripheral neuropathy per se as well as diabetic peripheral neuropathy.

Similarly, Jianbo et al. (2011) demonstrated that plasma concentration of total homocysteine was associated with diabetic neuropathy independent of the traditional risk factors.

Luo et al. (2013) demonstrated the presence of elevated plasma homocysteine in the absence of any other identifiable aetiology in a group of patients with peripheral neuropathy. They termed this condition IHIN (isolated homocysteine-induced neuropathy). Electrophysiological studies suggested large fibre neuropathy with demyelination and axonal denervation.

Shandal and Luo (2016) demonstrated that sensory deficits were the predominant components of IHIN.

Further studies are required to better understand this entity and improve its management.

5.8 Down's Syndrome

The first clinically identified human syndrome that was shown to be of chromosomal origin was Down's syndrome. Being trisomy 21, it bears a relation to homocysteine metabolism as the gene coding the transsulfuration pathway enzyme, CBS, resides on this chromosome. The phenotype of Down's syndrome

is, hence, opposite to that of homocysteinemia, which is attributed to the extra copy of the CBS gene present on the third chromosome 21. The metabolic consequence, as demonstrated by Pogribna et al. (2001), is that more homocysteine is directed to the transsulfuration pathway and less goes through the remethylation cycle. This results in a functional folate deficiency state. There is also hypoactivity of methionine synthase secondary to the removal of its substrate (homocysteine) by CBS.

As a consequence, there is less regeneration of methionine from homocysteine and less SAM, which are important for appropriate protein synthesis required especially for the processes of growth, immunity and hormone synthesis. Thus, there is a consistent milieu of hypomethylation due to the decreased synthesis of SAM and SAH. It has been suggested that this may contribute to the pathology of Down's syndrome.

In 1997, Yu et al. (1997) analysed chromosome 21 in patients with Down's syndrome. They found a high proportion of densely methylated interspersed repetitive sequences. These have been postulated to be a compensatory mechanism for down-regulation of the overexpressed genes on this chromosome.

In 2014, Nandha Kumar et al. (2014) studied the association of homocysteine and folate levels in controls as well as 108 children with Down's syndrome with or without congenital heart defects and neural tube defects. They observed that the homocysteine was significantly lower in the children with Down's syndrome as compared to the controls. At the same time, those with congenital heart defects had homocysteine levels that were significantly higher than in those without these defects. Homocysteine was lower in those with neural tube defects than those without these defects, but this was not significant. Folate levels were lower in the presence of both types of defects—congenital heart defects and neural tube defects—but this too was not significant. Hence, they concluded that the congenital heart defects seen in Down's syndrome could be a result of pathology due to altered homocysteine metabolism.

5.9 Psychiatric Disorders

So far, we have been talking about the effects of homocysteinemia. However, Levine et al. (2008) demonstrated the effect of post-traumatic stress disorder (PTSD) on circulating levels of homocysteine. They included 28 male patients of PTSD and compared them to 223 controls. The increased level of homocysteine was significant ($p < 0.001$); on applying logistic regression analysis to the data, the duration of PTSD was able to predict the homocysteine.

Psychiatric disorders differ from neurological disorders in that the former are mostly a sequel of the social environment, whereas the latter is the result of some somatic pathology. Consequently, the former is unaccompanied by physical signs like stroke, haemorrhage, etc. whereas the latter has definite physical signs and symptoms.

5.9.1 Neuropsychiatric Disorders

Neuropsychiatric disorders are a group of psychiatric conditions that are attributable to altered pathophysiology in the nervous system. Even though these disorders are likely to have multiple genetic and environmental causes, addressing each causative factor would result in improved management.

5.9.2 Schizophrenia

As described above, homocysteinemia has a multipronged effect on neurobiology, and it blocks the normal neuroinhibitory impulses mediated by GABA. The neuropathology of some neuropsychiatric disorders has been identified, e.g. schizophrenia is associated with oxidative stress and hypoactivity of the *N*-methyl-D-aspartate (NMDA) receptors in the brain. Glutamate interacts with these receptors and mediates postsynaptic excitation of neural cells. Homocysteine is known to interact with glutamatergic transmission in the brain. It stimulates the NMDA receptors leading to an increased influx of calcium into the neurons which culminates in neurotoxicity and apoptosis (Ho et al. 2002). As shown in Fig. 5.1, homocysteine also causes oxidative stress and aberrant DNA methylation. This would explain the implication of homocysteinemia in *schizophrenia*.

In the presence of low glycine levels, homocysteine acts as an antagonist within the glycine site of the NMDA receptors, thus exhibiting a neuroprotective effect at normal concentrations, but still being toxic at higher concentrations (Lipton et al. 1997). Alternately, in the presence of high glycine levels (as occurs in head trauma and stroke), even low homocysteine becomes toxic because it acts in synergism with the glycine (Alam et al. 1998). Thus, homocysteine has a dual effect on these receptors.

5.9.3 Depression

The possible connection between depression and homocysteine-methyl donor pathways was first described by Reynolds and his colleagues in the early 1970s and 1980s (Reynolds et al. 1970, 1984; Reynolds and Stramentinoli 1983). Several subsequent population studies reported high homocysteine in depressive disorders. In a meta-analysis, Bressa (1994) demonstrated that *S*-adenosyl methionine (SAM) functioned as an antidepressant, showing that mood can be altered by alterations in the homocysteine pathway.

Here, one would like to mention the two studies that did not find a correlation between homocysteine and depression. One study included 478 non-depressed, 100 mildly depressed and 122 severely depressed women. All three categories of subjects exhibited vitamin B_{12} deficiency (14.9%, 17% and 27%, respectively) but showed no association between homocysteine and depression (Penninx et al. 2000). In the other study, those with a lifetime diagnosis of major depression exhibited significantly lower serum and RBC folate levels as compared to those without

depression, but there was no association between homocysteine and depression (Morris et al. 2003).

Later, studies established that depressive episodes may predict the development of cardiovascular disease. It was proposed that since homocysteinemia is one of the risk factors of cardiovascular disease, it may be the co-occurring metabolic disruption between this condition and depression (de Jonge et al. 2014). The Rotterdam study of older men and women observed that high homocysteine and deficiencies of B_{12} and, to a lesser extent, of folate were associated with depression (Tiemeier et al. 2002). Similarly, the Hordaland study of Norway elucidated that in older men and women, high homocysteine coupled with the T/T allele of the MTHFR gene was associated with depression (Bjelland et al. 2003). Yapislar et al. (2012) observed raised levels of homocysteine and platelet aggregation along with low levels of nitric oxide in patients of panic disorder and major depressive disorders, indicating the role of homocysteine and its mechanism of action in these disorders. Ford et al. (2013) demonstrated that the memory and cognitive disabilities observed in depression were due to homocysteinemia.

5.9.4 Bipolar Disorder

Two common polymorphisms in the *MTHFR* gene (C677T and A1298C), which have been associated with schizophrenia, may also increase the risk of bipolar disorder. A meta-analysis was conducted associating MTHFR polymorphisms with the major psychiatric disorders (schizophrenia, bipolar disorder and unipolar depressive disorder). 29,502 subjects with MTHFR C677T and 7934 subjects with MTHFR A1298C were included. It was elucidated that all these psychiatric disorders shared a vulnerability due to the MTHFR 677TT genotype with an odds ratio of 1.26 for the TT versus the CC genotypes (Peerbooms et al. 2011). Another study on 120 patients of bipolar disorder examined the MTHFR and the CBS gene polymorphisms. They found an association of bipolar disorder with the CBS T833C genotype but not with the MTHFR C677T genotype (Permoda-Osip et al. 2014).

A point worthy of note is that patients of bipolar disease may have higher circulating levels of homocysteine if treated with sodium valproate or lamotrigine. The former has been found to inhibit methionine adenosyltransferase and the latter to weakly inhibit dihydrofolate reductase—both resulting in reduced functional folate levels despite normal folate levels in circulation (Baek et al. 2013).

Lacunae in Knowledge

Though more data is required in all aspects of neurological effects of homocysteinemia, two areas need more attention from researchers: (a) whether food fortification or early institution of vitamin supplements in the elderly age group would reduce the incidence/progress of AD and dementia and (b) whether there is a therapeutic role of vitamins in neuropsychiatric disorders.

Clinical Message

1. Since homocysteinemia and folate deficiency are implicated in many neural tube defects and autism, these should be measured in all prospective mothers before as well as during pregnancy so that timely action may be taken to avoid these defects. Ideally, periconceptual folate supplements are recommended. At the same time, vitamin B_{12} status must also be ascertained and the vitamin supplemented if required.
2. It would be pertinent to advise measurement of circulating homocysteine in all cases of stroke, cerebrovascular accident, cognitive impairment, dementia, Alzheimer's disease, epilepsy and peripheral neuropathy especially diabetic neuropathy. In fact, circulating homocysteine should be measured in all elderly patients along with vitamin B_{12} and folate.
3. If these levels are elevated, then treating with an appropriate vitamin supplement (preferably containing all three modulating vitamins—folate, pyridoxine and vitamin B_{12}) would enable the clinician to reduce the morbidity by eliminating one confounding factor.
4. Diagnosing AD or MCI is just the tip of the iceberg; preventing further cognitive decline or, better still, improving cognition is the current therapeutic target. Keeping a control on the circulating levels of homocysteine could be one step towards achieving that target.
5. Equally pertinent would be the measurement of plasma homocysteine in Parkinson's disease with the aim of reducing homocysteine if it is found elevated to avoid comorbidities. Even better would be the concomitant administration of tolcapone to patients receiving L-DOPA as treatment for PD.
6. Also, since anticonvulsants are a heterogenous class of drugs with a common ability to cause folate deficiency by a variety of mechanisms (e.g. reduced intestinal absorption, increased metabolism of folates in liver, altered activity of some enzymes involved in one-carbon transfer, etc.), adding folate to the anticonvulsant regimen would be advisable (Lambie et al. 1985).

7. In Down's syndrome, there is no homocysteinemia—the contrary, in fact. However, there is a functional deficiency of folate. Hence, it may be prudent to modify the nutrition in these subjects by supplementing two substances: (a) methionine-rich diet (to promote the production of SAM and SAH) and (b) folinic acid (to bypass the folate cycle).
8. It is evident that homocysteinemia plays a role even in the aetiology of neuropsychiatric disorders. Moreover, drugs used for some of these conditions cause homocysteinemia by various mechanisms. Hence, circulating homocysteine levels should be monitored in these patients, too, and homocysteine-lowering therapy given to them to decrease the probability of comorbidities.
9. In addition, it would be:
   (a) Beneficial to start vitamin B supplements in the elderly so as to prevent homocysteinemia-induced changes, thereby minimizing cognitive decline
   (b) Advisable to ensure periconceptual intake of folate as well as B_{12} to prevent NTDs and autism