Original Article

Clinical, Biochemical, Radiological, and Genetic Profile of Patients with Homocysteine Remethylation Pathway Defect and Spastic Paraplegia

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Abstract

Objectives: The objective of this study is to describe the clinical, biochemical, radiological, and genetic profile of patients presenting with progressive spastic paraparesis due to homocysteine remethylation pathway defect. **Methods:** This was a retrospective study conducted by reviewing the medical records of patients with serum homocysteine levels >50 µmol/L between January 2015 and January 2019 at our hospital. We included patients presenting with progressive spastic paraparesis, having serum homocysteine >50 µmol/L with low or normal blood methionine suggesting disorders of homocysteine remethylation. Demographic details, clinical manifestations, biochemical abnormalities, neuroimaging findings, and genetic profile were analyzed. **Results:** A total of seven patients (M: F = 5:2) fulfilled the study eligibility criteria. The mean age at onset of the disease was 13.4 ± 2.4 years (range: 9–17 years). Spastic paraparesis was the presenting manifestation in 4/7 (57.1%) patients. Other manifestations included cognitive decline, poor scholastic performance, behavioral disturbances, seizures, and spastic bladder. Severe hyperhomocysteinemia (>100 µmol/L) was noted in 6/7 (85.7%) patients with median levels of serum homocysteine being 185.7 µmol/L (range: 85.78–338.5 µmol/L). Neuroimaging showed parieto-occipital predominant leukoencephalopathy in 5/7 (71.4%) and diffuse cerebral atrophy in 1/7 (14.2%). Genetic analysis in three patients revealed pathogenic missense variants c.459C >G (p.Ile153Met), c.973C >T (p.Arg325Cys), and c.1031G >T (p.Arg344Met) in *MTHFR* gene. All the patients received vitamin B12 (injection and oral), folic acid, and pyridoxine and two patients received betaine. At the last follow-up of a median duration of 12 months, there was a good clinical and biochemical response with reduction in the median value of serum homocysteine by 77.5 µmol/L. **Conclusion:** Evaluation of serum homocysteine and blood methionine in adolescents presenting with progressive spastic paraparesis gives clue to a treatable homocysteine remethylation disorders.

Keywords: Hyperhomocysteinemia, metabolic myelopathy spastic paraparesis, *MTHFR* (methylenetetrahydrofolate reductase) gene, remethylation disorders

Introduction

Homocysteine (Hcy) is a sulfur-containing amino acid produced in the body through essential amino acid methionine (Met).[1] Hcy is metabolized to Met through remethylation and cystathionine through trans-sulfuration. The remethylation of Hcy requires enzymes Met synthase (*MTR* gene; CblG defect), Met synthase reductase (*MTRR* gene; CblE defect), and methyl group transfer from 5-methyl tetrahydrofolate with methylcobalamin as a cofactor.[1] The enzyme methylenetetrahydrofolate reductase (MTHFR) helps in replenishing 5-methyl tetrahydrofolate by catalyzing the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate.[1] Hcy remethylation also occurs independently in the liver from the conversion of betaine to dimethylglycine by betaine Hcy methyltransferase.[1] The remethylation of Hcy prevents the toxic accumulation of it in the brain, heart, eyes, and skeletal system. Biochemically increased serum total Hcy with low or normal serum Met differentiates disorders of remethylation from trans-sulfuration.[2] Remethylation pathway defects can present across the age group from infancy to adulthood and manifestations are diverse.[2,3] Combined and isolated disorders of remethylation usually present in neonates or infants with poor feeding, failure to thrive, encephalopathy, seizures, muscular hypotonia, and visual impairment with hematological manifestations like

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megaloblastic anemia or pancytopenia.[2-4] Older children and adults manifest with neurocognitive impairment, visual impairment, and features of subacute combined degeneration of the cord.[5] Thromboembolism is less likely unlike classical homocystinuria.[2,3] MTHFR deficiency manifests similarly in adolescents and adults but without hematological manifestations.[6] Other manifestations of remethylation disorders include hemolytic uremic syndrome, cardiomyopathy, pulmonary hypertension, and growth failure.[2,3] Spastic paraparesis due to remethylation disorders usually seen in adolescents or adults is easily amenable to treatment though is usually misdiagnosed. Published literature on disorders of Hcy remethylation is limited to anecdotal case reports and short case series.[7-12] There is a paucity of literature from India regarding progressive spastic paraplegia due to Hcy remethylation pathway defect. Here, we describe the clinical, biochemical, radiological, and genetic profile of patients presenting with progressive spastic paraparesis due to hyperhomocysteinemia secondary to remethylation pathway defects.

Subjects and Methods

Study design

The study was a retrospective study done in a quaternary care center for neurological disorders in South India. The hospital case records were reviewed of the patients with elevated serum Hcy levels, which was defined as serum Hcy level of >50 μmol/L, who were under the care of the authors and evaluated at our hospital between January 2015 and January 2019. From these hospital case records, we included for this study patients fulfilling the following inclusion criteria: (i) patients presenting with progressive spastic paraparesis, (ii) had elevated serum Hcy levels (>50 μmol/L), and (iii) had low or normal blood Met levels, thereby suggesting disorders of Hcy remethylation. Exclusion criteria were patients with secondary causes of spastic paraparesis like compressive myelopathy, demyelination, arginase deficiency, biotinidase deficiency, leukodystrophy, and cerebral or spinal cerebrovascular accidents.

Data collection

Following data were collected: demographic details, clinical features, family history of similar illness, consanguinity, and clinical examination findings. Complete blood count, peripheral smear, blood indices, serum levels of vitamin B12, folate, and Hcy were noted. Serum vitamin B12, folate, and Hcy were measured by electrochemiluminescence immunoassay using an automated immunoassay analyzer (Cobas 6000, Roche, Germany). Blood amino acids, free carnitine, and a panel of acylcarnitines were measured by electrospray-ionization tandem mass spectrometry. Magnetic resonance imaging (MRI) of the brain and spine were analyzed by an independent and qualified neuroradiologist blinded to the clinical details if the images were available on picture archiving and communication system. Targeted exome sequencing was employed for detection of genetic variants. Sequencing was performed on the Illumina NextSeq550 platform at our institute. In-house bioinformatic pipeline was employed for raw data analysis, variant calling, and annotation. Other laboratory parameters noted include urinary organic acids, renal function tests, liver function tests, serum ammonia, and serum lactate.

Results

A total of seven patients (five males and two females) fulfilled our inclusion criteria and were selected for detailed analysis. The demographic and clinical manifestations are summarized in Table 1. The biochemical parameters, brain and spine imaging, and genetic analysis have been summarized in Table 2.

Clinical features ($n = 7$)

Six patients had presented in the second decade of life, one patient in the late first decade. The mean age at onset of the disease was 13.4 ± 2.4 years (range: 9–17 years), and the mean age at initial presentation was 15.6 ± 2.4 years (range: 11–18 years 6 months). There was a median delay of 24 months in the diagnosis. The initial manifestation was gait disturbance (spastic gait) with recurrent falls in four patients (57.1%). The other three patients had behavioral disturbances (14.3%), recurrent encephalopathy (14.3%), and seizures (14.3%) each as presenting manifestation. Other associated clinical features noted are listed in Table 1. Consanguinity was present in only one patient. On general examination hyperpigmentation of the face, hands were seen in one patient; short, hypopigmented hairs in one patient; and marfanoid habitus in two patients. Spasticity predominantly involving the lower limbs was present in all seven patients with exaggerated muscle stretch reflexes and extensor plantar response. According to modified Medical Research Council grading, the muscle power in all the patients was 4/5 to 5/5 in the lower limbs proximally and normal in the upper limbs. Impaired joint position sense and vibration sense were noted in two patients (28.6%).

Biochemical parameters ($n = 7$)

The serum Hcy level was above 100 μmol/L in six patients. The median serum Hcy level was 185.7 μmol/L (range: 85.78–338.5 μmol/L). Qualitative urine homocystine was positive in one patient whose serum Hcy was 338.5 μmol/L. The median blood Met was 12.2 μmol/L (range: 4.75–16.0 μmol/L) which was within normal limits. The median blood propionyl carnitine (C3) was 1.66 μmol/L (range: 0.55–1.89 μmol/L). Urinary organic acids available in two patients showed no abnormality. Serum vitamin B12 level was below the normal range in six patients. The median serum vitamin B12 levels were 188 pg/mL (58–230 pg/mL). Serum folate level was low in two patients with a median of 5.22 ng/mL (1.88–10.95 ng/mL). The peripheral smear showed normocytic normochromic blood picture in all patients. Serum ammonia, serum lactate, renal function, and liver function tests were normal in all the patients. Patient number three had severe osteopenia on lumbar and cervical spine X-ray [Figure 1]. The bone mineral density (BMD) measured at anteroposterior spine

L1–L4 was 0.594 g/cm^2 with age match *Z* score being −2.5 *Z* score and BMD measured at total body was 0.774 g/cm^2 with age match *Z* score being −2.0 *Z* score. Nerve conduction studies showed sensorimotor axonal polyneuropathy in two patients.

Neuroimaging findings (*n* = 7)

Cranial MRI images were available in all patients and MRI spine was available in six patients. Cranial MRI in four patients showed T2/fluid-attenuated inversion recovery (FLAIR) hyperintensities involving periventricular white matter of bilateral frontal, parieto-occipital regions, and centrum semiovale [Figure 2]. In one patient, T2/FLAIR hyperintensities were noted in the peri-trigonal regions of both hemispheres along with diffuse brain atrophy [Figure 2 P3a, 3b]. Parieto-occipital predominance of white matter hyperintensities was noted in all five patients. The signal changes were asymmetric in three patients (right >left). No areas of blooming or restricted diffusion were noted on susceptibility-weighted imaging and diffusion-weighted imaging, respectively. There was no contrast enhancement in any of the scans. The cranial MRI was normal in two patients. Spinal MRI was normal in all the subjects.

Mutation analysis (*n* = 3)

Genetic analysis was available in three patients and all of them carried mutations in the *MTHFR* gene (NM_005957.5) [Figure 3]. Patient 1 and Patient 2 carried homozygous missense variation in exon 3 which results in the amino acid substitution of Met for isoleucine at codon 153 c. 459C >G (p.Ile153Met). This variant has not been reported in the 1000 genomes database and has a minor allele frequency of 0.01% in the ExAC and 0.09% from individuals of South Asian background in gnomAD but never in homozygous state. There is a small physicochemical difference between isoleucine and Met. Three variants within six amino acid positions of the variant p.Ile153Met have been shown to be pathogenic, while none have been shown to be benign. The in-silico predictions of the variant are probably damaging by PolyPhen-2 and damaging by SIFT, LRT, and MutationTaster2. The isoleucine residue at codon 153 of MTHFR is conserved in all mammalian species. The nucleotide c. 459 in MTHFR is predicted to be conserved by GERP++ and PhyloP across 100 vertebrates. For these reasons, this variant was classified as pathogenic.

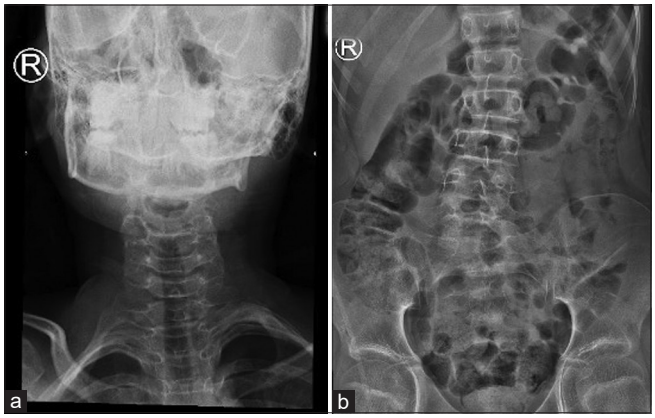

Figure 1: (a) A–P radiograph of cervical spine showing diffuse osteopenia of vertebrae with reduced height. (b) A–P radiograph of lumbosacral spine demonstrating severe osteopenia of lumbar vertebrae with reduced height and kyphoscoliosis. The pelvic bones also appear osteopenic

Patient 3 carried missense mutations in compound heterozygous state in exon 6 of *MTHFR*. The first variant c. 973C >T (p. Arg325Cys) that results in the substitution of arginine with cysteine in 5,10-methylenetetrahydrofolate reductase protein at amino acid position 325 has been reported previously as pathogenic in human gene mutation database (HGMD).[13] There is a large physiochemical difference between arginine and cysteine, which is likely to impact secondary protein structure as these residues differ in polarity, charge, size, and/or other properties. The arginine residue at codon 325 of MTHFR is conserved in all mammalian species. The nucleotide c. 973 in MTHFR is predicted conserved by GERP++ and PhyloP across 100 vertebrates. This variant is predicted to be damaging by both SIFT and PolyPhen2. The p.Arg325Cys variant is observed in 0.0065% alleles from individuals of South Asian background in gnomAD exomes and in 0.0994% alleles from individuals of European background in 1000 genomes but never in homozygous state. This variant is absent in our in-house database. As the phenotype of the proband matches with that of the p.Arg325Cys missense variant, this variant has been classified as likely pathogenic. The second variant, c. 1031G >T (p.Arg344Met), results in the substitution of argininewith Met in the protein at amino acid position 344. c. 1031G >T has not been reported previously in literature nor in gnomAD, 1kG, and in our in-house database. The p.Arg344Met variant is novel (not in any individuals) in gnomAD, 1kG as well as in our in-house database. There is a moderate physicochemical difference between arginine and Met. Three variants within six amino acid positions of the variant p.Arg344Met have been shown to be pathogenic, while none have been shown to be benign. The p.Arg344Met missense variant is predicted to be damaging by both SIFT and PolyPhen2. The nucleotide c. 1031 in MTHFR is predicted conserved by GERP++ and PhyloP across 100 vertebrates. For these reasons, this variant has been classified as likely pathogenic.

Treatment and Follow-up (*n* = 7)

All the patients received injection hydroxocobalamin intramuscularly (1 mg), oral pyridoxine (100 mg), folic acid (5 mg), methylcobalamin (1500 mcg), and baclofen daily. Inj. hydroxocobalamin was advised initially daily for 2 weeks, followed by once fortnightly for a month, and once monthly maintenance. Two patients who were able to procure betaine received it at 250 mg/kg/day (around 6 g/day). All the patients who were available for follow-up reported improvement in spasticity, and one patient also had improvement in behavioral symptoms. Patient five was lost to follow-up. The median serum Hcy was 108.2 µmol/L (range: 52.74–188.8 µmol/L) at the last follow-up with a median follow-up duration of around 12 months (range 3–24 months).

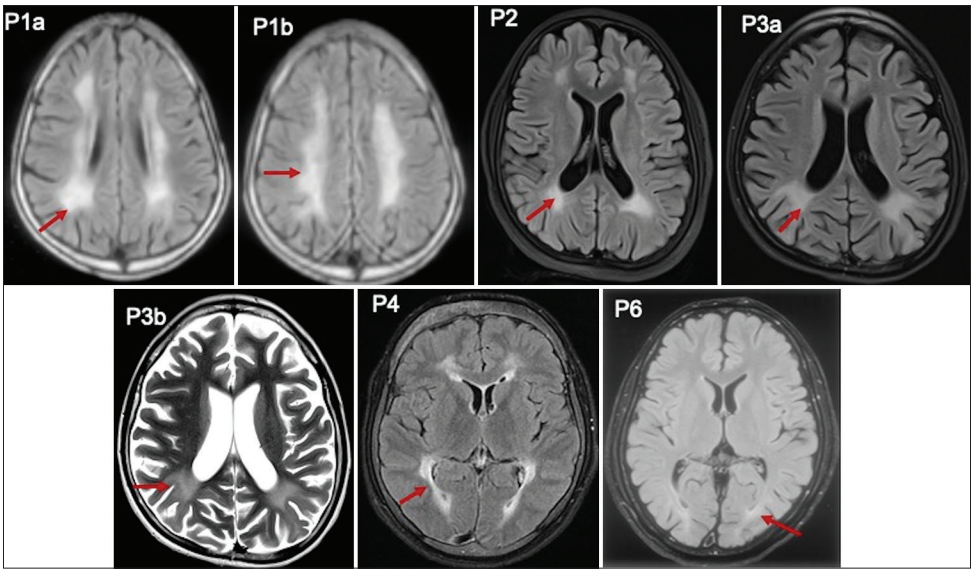

Figure 2: P (patient); P1a, P2, P4 axial FLAIR image demonstrating symmetric anterior and posterior periventricular hyperintensities; P1b axial FLAIR image showing diffuse hyperintensities in the centrum semiovale; P3a, P6 axial FLAIR image demonstrating symmetric hyperintensities in the periventricular white matter in the parietal regions; and P3b Axial T2 image showing diffuse cerebral atrophy along with signal changes in the peritrigonal white matter

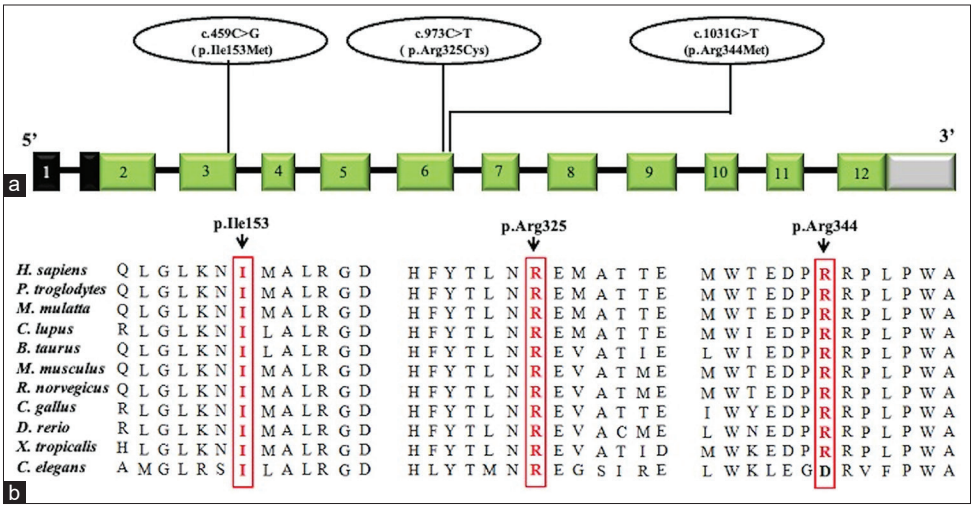

Figure 3: (a) Schematic representation of the mutations and their location on MTHFR observed in this study. Green rectangular boxes represent coding exons, black boxes represent 5′ noncoding region, and gray boxes mean 3′ noncoding region. Introns are represented by dark lines. Exon numbers are represented inside the boxes. The size of exons/introns is not represented at scale. (b) Conservation of amino acid residues across species for the three coding mutations detected in our study

Discussion

Chronic slowly progressive spastic paraparesis can be an independent or accompanying manifestation of various inherited or acquired disorders affecting the corticospinal tract. The acquired causes of progressive spastic paraparesis include compressive myelopathy due to various causes, central nervous system demyelinating diseases like multiple sclerosis, nutritional deficiencies like vitamin B12, and folate deficiency. The inherited/genetic causes include Hcy remethylation defects, urea cycle defects like arginase deficiency, triple H syndrome, biotinidase deficiency, nonketotic hyperglycinemia, peroxisomal disorders like adrenoleukodystrophy, lysosomal storage disorders like Krabbe disease, metachromatic leukodystrophy, and hereditary spastic paraplegia.[14] Our study describes the features of seven patients presenting with progressive spastic paraparesis due to Hcy remethylation pathway defects. A literature review on the patients presenting with spastic paraparesis due to remethylation pathway defects has been summarized in Table 3.

Similar to the patients described by Wei *et al.*[11] and Chang *et al.*,[12] most of our patients were adolescents (11–19 years) presenting subacutely with progressive spastic paraparesis. One patient had an acute presentation with recurrent encephalopathy later progressing to develop spastic gait. Other manifestations included cognitive decline, poor

scholastic performance, behavioral disturbances, seizures, gait ataxia, and spastic bladder. These patients were initially considered to have either adrenoleukodystrophy in view of parieto-occipital leukoencephalopathy on neuroimaging or hereditary spastic paraplegia. Non-neurological manifestations included marfanoid habitus (14.3%) and optic atrophy (28.6%). Biochemically, the presence of hyperhomocysteinemia with low or normal levels of Met suggests a defect in Hcy remethylation pathway either due to genetic defects in the enzymes involved in this pathway or due to the deficiency of the vitamin cofactors (vitamin B12, folate).[1,2] All the patients in our study had serum Hcy >50 µmol/L with 86% of them having severe hyperhomocysteinemia (>100 µmol/L), 14% had intermediate (31–100 µmol/L), and 100% had normal Met consistent with disorders of Hcy remethylation.[1,2] Genetically mediated remethylation pathway defects have been described to occur due to disorders of intracellular cobalamin transport affecting methylcobalamin (cblD-Hcy, cblE, and cblG) or both methylcobalamin and adenosylcobalamin (cblC, cblD-MMA/Hcy, cblF, and cblJ), or due to the deficient production of *MTHFR* enzyme.[2,3] All the patients in our study had normal levels of blood propionyl carnitine indirectly suggesting normal levels of blood methylmalonic acid, thus excluding disorders of combined methylmalonic acidemia and homocystinuria. Hence, our cohort primarily composed of patients either with isolated remethylation disorders (cblD-Hcy, cblE, and cblG) and those with *MTHFR* deficiency.

Serum vitamin B12 levels were low in six patients with no evidence of megaloblastic blood picture and were normalized after treatment with inj. hydroxocobalamin. Three out of six patients with low serum B12 were later genetically confirmed to have mutations in the *MTHFR* gene. Additionally, three patients with low B12 with no genetic testing had persistent high serum Hcy level despite normalization of B12, so clinically and biochemically belong to Hcy remethylation defect. Thus, persistent elevation of serum Hcy despite normalization of B12 levels points toward non-nutritional or genetically mediated cause for hyperhomocysteinemia. Serum folate was low in two patients who had *MTHFR* deficiency corroborating with the diagnosis.

The presence of severe osteopenia and osteoporosis in Patient 3 is one of the less described manifestations of remethylation pathway defects. Skeletal abnormalities are well described in

Table 1: Demographic and clinical profile of the seven patients with spastic paraparesis secondary to homocysteine remethylation pathway defect

Patient No	1	2	3	4	5	6	7
Age at onset	13 years	13 years	9 years	13	16 years	17 years	15 years
Age at presentation	13 years 8 months	16 years	11 years	17 years	17 years	18 years 6 months	17 years 4 months
Sex	Male	Female	Female	Male	Male	Male	Male
Presenting symptom	Recurrent falls and gait impairment	Two episodes of encephalopathy followed by recurrent falls and gait impairment	Difficulty in getting up from squatting posture and behavioral disturbances	Recurrent falls and gait impairment	Recurrent falls and gait impairment	Recurrent falls and gait impairment	Seizures and gait impairment
Other Clinical Features							
Seizures	-	-	-	-	-	-	+
Poor Scholastic Performance	-	-	+	+	+	-	-
Cognitive Decline	+	+	+	+	-	+	-
Behavioral Disturbances	-	+	+	+	-	-	-
Gait Ataxia	-	+	-	-	-	-	-
Bladder disturbances	-	Urgency	-	-	-	-	-
Consanguinity	+	-	-	-	-	-	-
Examination findings							
General Examination	Hyperpigmentation (Face, Hands)	-	Hypopigmented hairs	-	-	-	-
Marfanoid Habitus	-	-	+	-	-	-	+
Fundus	Normal	Normal	Normal	Temporal disc pallor	Normal	Temporal disc pallor	Normal
Spasticity	+ (Lower limbs)	+ (Lower limbs)	+ (Lower limbs) with scissoring	+ (Lower limbs)	+ (Lower limbs) with scissoring	+ (Lower limbs)	+ (Lower limbs)
Reflexes	Exaggerated	Exaggerated	Exaggerated	Exaggerated	Exaggerated	Exaggerated	Exaggerated
Plantar	Extensor	Extensor	Extensor	Extensor	Extensor	Extensor	Extensor
Impaired joint position and vibration sense	-	+	+	-	-	-	-

Table 2: Biochemical, radiological and genetic profile of the seven patients with spastic paraparesis secondary to homocysteine remethylation pathway defect

Patient No	1	2	3	4	5	6	7
Serum Homocysteine at diagnosis (<15 µmol/L)	197.3	145.3	338.5	185.77	85.78	176.04	209.01
Blood Methionine (3-44 µmol/L)	8.6	12.23	4.75	13.49	16.01	12.23	7.77
Blood Propionylcarnitine, C3 (0.30-5.81 µmol/L)	0.55	1.66	0.7	1.31	1.89	1.68	1.66
Serum Vitamin B12 (197-771pg/mL)	194.7	230	194	58	70	62	188
Serum Folate (3.1-19.9 ng/mL)	N/A	2.27	1.88	6.79	7.99	3.66	10.95
MRI Brain	Periventricular Leukoencephalopathy (Posterior Predominant)	Periventricular Leukoencephalopathy (Posterior Predominant)	Periventricular Leukoencephalopathy (Posterior Predominant) Diffuse cerebral atrophy	Periventricular Leukoencephalopathy (Posterior Predominant)	Normal	Periventricular Leukoencephalopathy (Posterior Predominant)	Normal
MRI Spine	Normal	Normal	Normal	Normal	Normal	Normal	N/A
Serum Homocysteine at last follow up (<15 µmol/L)	127.2	89.2	65	52.74	N/A	188.8	151
Follow up duration in months	24	4	3	22	N/A	15	9
Genetic Analysis	*MTHFR* *Exon 3* Homozygous c. 459C>G (*p.Ile153Met*)	*MTHFR* *Exon 3* Homozygous c. 459C>G (*p.Ile153Met*)	*MTHFR gene* *Exon 6* Compound Heterozygous c. 973C>T (p.Arg325Cys); c. 1031G>T (p.Arg344Met)	N/A	N/A	N/A	N/A

*N/A not available

Table 3: Summary of the published studies of patients presenting with spastic paraparesis secondary to disorders of homocysteine remethylation

Authors	Number of patients reported	Age of onset	Biochemical Phenotype	Radiological findings	Genetic analysis
Bathgate D *et al.*[7] (2012)	2	Young adults	Serum homocysteine: 155-163 µmol/L (5-15 µmol/L) Serum methionine: 15-18 µmol/L (15-40 µmol/L)	MRI Brain: mild cortical atrophy MRI Spine: normal	Both siblings had homozygous mutations in the MTHFR gene (c596C>T, pA195V)
Lossos A *et al.*[8] (2014)	4	30-45 years	Serum homocysteine: 140-185 µmol/L (5-15 µmol/L) Serum methionine: 0.075-0.16 mg/dl (20-35 mg/dl)	MRI Brain: diffuse cerebral atrophy (4/4) Leukoencephalopathy, mainly posterior periventricular (4/4) Spinal cord atrophy (4/4)	Mutations were detected in the MTHFR gene Patient 1 and 2: c. 1141C>T (p. W381R); c. 1535A>G (p.Y512C) Patient 3 and 4: c. 1130G>A (p. R377H)
Lin N *et al.*[9] (2016)	1	38 years	Serum homocysteine: 269.8-313.7 µmol/L Serum methionine, methylmalonic acid, B12, and folic acid - normal	MRI Brain: posterior leukoencephalopathy	Mutations were detected in the MTHFR gene Mutation 1 :c. 1699C>T Mutation 2 c. 698C>G
Perna A *et al.*[10] (2018)	2	23-27 years	Serum homocysteine: 146-176 µmol/L (5-15 µmol/L) Serum methionine: 13.1-14.3 µmol/L (15-54 µmol/L) Urine homocysteine: 12.2-16.2 (normally should be absent)	MRI Brain: symmetric posterior leukoencephalopathy	Compound heterozygous mutations in the MTHFR gene were detected in both the siblings c. 1320G>A; c. 237-2A>G
Wei Y *et al.*[11] (2019)	8	7-26 years	Serum homocysteine: 75.4-250.1 µmol/L (5-15 µmol/L) Serum propionylcarnitine: 7.06-12.31µmol/L (0.5-4 µmol/L) Serum methylmalonic acid: 0.115-0.926 mg/dl (≤ 0.047) Urine methylmalonic acid: 44.1-1731.4 times higher than control values of same age group	MRI brain showed mild atrophy in 6/8 patients MRI Spine was normal in all	Definite pathogenic compound heterozygous mutations in the MMACHC gene were detected in 5/8 patients Patient 1: c. 482G>A; c. 609G>A Patient 3: c. 394C>T; c. 565C>A Patient 5: c. 482G>A; c. 658_660 del Patient 6: c. 914 T>C; c. 278G>A Patient 7: c. 482G>A; C.656_658 del
Chang KJ *et al.*[12] (2020)	7	14-40 years	Serum homocysteine: 53.1-154.5 µmol/L (5-15 µmol/L) Urine methylmalonic acid: Patient 1-5: 142.4-332.9 mmol/mol creatinine (0.2-3.6 mmol/mol creatinine) Patient 6-7: 2.2-2.6 mmol/mol creatinine (0.2-3.6 mmol/mol creatinine)	Patient 1: Reversible cerebellar changes, delayed/impaired myelination, periventricular hyperintensity Patient 2: periventricular hyperintensity Patient 6: delayed/ impaired myelination Normal in 4/7 patients	Compound heterozygous mutations were detected in MMACHC gene (Patient 1-5) and MTHFR gene (Patient 6-7) Patient 1: c. 482G>A, c. 658-660delAAG Patient 2: c. 482G>A, c. 609G>A Patient 3: c. 482G>A, c. 609G>A Patient 4: c. 482G>A, c. 658-660delAAG Patient 5: c. 482G>A, c. 658-660delAAG Patient 6: c. 136C>T, c. 698C>G Patient 7: c. 136C>T, c. 236+1G>A

classical homocystinuria and attributed to the high levels of serum Hcy (>100 µmol/L). Increased serum Hcy regulates bone remodeling by increasing the activity of osteoclasts, inducing apoptosis of osteocytes and osteoblasts, and by impeding osteoblastic differentiation leading to increased bone resorption and decreased bone formation.[15] Skeletal abnormalities in Patient 3 can be ascribed to very high serum Hcy levels >300 µmol/L who was later genetically confirmed to have mutation in *MTHFR* gene.

The imaging abnormalities described in remethylation disorders include diffuse cerebral atrophy, white matter changes, basal ganglia lesions, and hydrocephalus.[15] The cranial imaging findings in our cohort were periventricular leukoencephalopathy with posterior predominance in 72%, cerebral atrophy in 14%, and normal in two patients. Leukoencephalopathy in remethylation pathway defects has been hypothesized to be secondary to interference in the process of myelination by the failure of methylation of myelin

basic protein.[16,17] Reversible leukoencephalopathy has been described in classical homocystinuria due to cystathionine beta-synthase deficiency and authors have hypothesized it to be secondary to increased levels of Met.[18,19] Wei *et al.*[11] have described only mild cerebral atrophy in eight cases of combined homocysteinemia with methylmalonic acidemia. Chang *et al.*[12] in his cohort have described periventricular hyperintensity/delayed myelination in 3/7 cases of remethylation pathway defects.

Genetic analysis in the first three patients revealed likely pathogenic missense variants in the *MTHFR* gene. Mutations in the *MTHFR* gene are the second most common cause of remethylation disorders next to cblC defect.[2,3,20] The MTHFR gene has a positive *Z* score (constraint score for tolerance to missense variants) of 0.9 suggesting that the rate of benign missense variants in the gene is rare. Further, the variants c. 459C >G (p.Ile153Met) and c. 973C >T (p.Arg325Cys) are in the catalytic domain of the enzyme, and the variant c. 1031G >T (p.Arg344Met) is in the linker domain, which are highly conserved. The regions in and around these three variants are hotspots for pathogenic variants with almost near absence of benign variants, further providing support to their pathogenicity. Literature is scarce in terms of genotype–phenotype correlation with respect to the previously reported variant p.Ile153Met. More data regarding functional analyses like residual enzyme activity or further case reports are required to derive at a meaningful genotype–phenotype correlation. Based on the biochemical evaluation 4/7 patients in our cohort whose genetic analysis was not available probably have either isolated remethylation disorders (cblD-Hcy, cblE, and cblG) or MTHFR deficiency.

Parenteral hydroxocobalamin which is a cofactor for Met synthase and betaine by using the alternate pathway for remethylation are the two proven modalities of treatment for patients with remethylation disorders.[2,3] All our patients received hydroxycobalamin and only two patients received betaine due to financial constraints. At the last follow-up of a median duration of 12 months, patients were symptomatically better with improvement in spasticity, gait, and behavioral symptoms, and biochemically, there was a reduction in the median value of serum Hcy by 77.5 µmol/L.

To the best of our knowledge, ours is the first Indian study to describe the cohort of spastic paraparesis secondary to Hcy remethylation pathway defects. Strengths of the study include a well-defined inclusion and exclusion criteria including subjects with only Hcy remethylation disorders; detailed description of the clinical manifestations, biochemical and imaging findings; and having a median follow-up of a year demonstrating a favorable clinical and biochemical response to treatment. The limitations of this study are its retrospective nature, small sample size, attrition of one patient, and availability of genetic confirmation in only three patients.

Conclusion

Hcy remethylation defects are one of the few treatable metabolic causes of progressive spastic paraparesis. A simple measurement of serum Hcy and blood Met in adolescents presenting with progressive spastic paraparesis with or without cognitive decline, poor scholastic performance, and behavioral disturbances and neuroimaging showing evidence of posterior predominant leukoencephalopathy gives clue to treatable disorders of Hcy remethylation.

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Conflicts of interest

There are no conflicts of interest.

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