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A study of Iraqi patients with homocysteine remethylation disorders in a tertiary pediatric centre

Mays R. Al-Tai [a,*], Adel A. Kareem [b], Nebal W. Saadi [c], Tawfig Ben Omran [d], Ban A. Abdul Majeed [e], Ibrahim F. Ibrahim [f], Lamia A. Alattar [g]

[a] Metabolic Unit, Children Welfare Teaching Hospital, Baghdad, Iraq
[b] Pediatric Neurology Unit, Children Welfare Teaching Hospital, Baghdad, Iraq
[c] College of Medicine, University of Baghdad, Pediatric Neurology Unit, Children Welfare Teaching Hospital, Baghdad, Iraq
[d] Hamad Medical Corporation, Doha, Qatar
[e] The Pioneer Molecular Pathological Lab., Baghdad, Iraq
[f] Aamal National Hospital for Cancer Management, Medical City Directories, Baghdad, Iraq
[g] Radiological Department, Children Welfare Teaching Hospital, Baghdad, Iraq

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ABSTRACT

Background: Hyperhomocysteinemia is a group of inherited homocysteine metabolism disorders characterised by elevated blood homocysteine levels (total homocysteine >15 μM). Homocystinuria is classified into two main homocysteine metabolism disorders. Classical Homocystinuria is caused by a deficiency of the pyridoxine-dependent enzyme cystathionine beta-synthase in the trans-sulfuration pathway. Non-classical Homocystinuria is a group of disorders affecting the interconversion of methionine to homocysteine through the re-methylation pathway.

Aim: This study aims to describe the clinical, biochemical, and genetic profiles of patients with re-methylation disorders.

Patients and methods: A cohort study was conducted at the metabolic clinic of Children Welfare Teaching Hospital in Baghdad from the 1st of December 2021 to the 1st of December 2022. The study included fifteen patients who met the following criteria: (1) elevated serum homocysteine levels (>15 μmol/L); (2) low or normal blood methionine levels (12–40 μmol/L). **Results:** fourteen MTHFR patients underwent statistical analysis, and one CblC patient was assessed separately. MTHFR patients comprised nine females and five males. The mean age at presentation was 7.1 years ±4.5, ranging from 1 to 16 years. Consanguineous marriages were reported in 13 patients. A family history of a similar disorder was documented in 73 % of cases. Among the families, four had two affected siblings. The two main reported clinical manifestations were gait disturbance (10/14, 71.4 %) and cognitive impairment/intellectual disability (6/14, 42.8 %). Brain MRI was conducted for all studied patients, with leukodystrophy being the most common finding (8/14, 57.1 %). Molecular testing revealed variants in *MTHFR* in 14 patients, and *MMACHC* in one patient.

Conclusion: According to this study, individuals with homocysteine re-methylation disorders can manifest symptomatology such as neuroregression, psychomotor delay, and whiter matter changes earlier than anticipated. And these disorders are amenable to treatment. Genetic testing is crucial in identifying the specific mutation type and guiding definitive treatment.

1. Introduction

Homocystinuria (HCU) comprises several disorders of homocysteine (HCy) metabolism shared by the common biochemical finding of a high blood HCy concentration. Hyperhomocystinaemia (total homocysteine (tHcy) >15 μM) can be caused by a nutritional deficiency of vitamins B12 or folate, mild chronic renal disease, and MTHFR polymorphisms or genetic factors, including inborn errors of metabolism [1]. Homocysteine is a sulfur amino acid not used for protein synthesis but synthesised from the essential amino acid methionine via transmethylation. It is

* Corresponding author.
E-mail address: Mays.altai@yahoo.com (M.R. Al-Tai).

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located at a branchpoint of two metabolic pathways, and these are the trans-sulfuration pathway in which the homocysteine is irreversibly degraded to cysteine by the action of pyridoxal 5′- phosphate (the active form of vitamin B6) - dependent enzymes: cystathionine β-synthase (CBS) and cystathionine γ-lyase. The other pathway is the *Re*-methylation pathway, in which the Homocysteine remethylated to methionine is catalysed by the methionine synthase enzyme using vitamin B12 as a cofactor which transfers a methyl group from 5-methyltetrahydrofolate (5-methylTHF) via cobalamin to homocysteine [2]. This reaction links the folate cycle with homocysteine metabolism [3]. Homocysteine re-methylation disorders are rare inherited disorders caused by deficient activity of the enzymes involved in the re-methylation of homocysteine to methionine. Elevated serum tHcy with low or normal methionine is their standard biochemical marker. The re-methylation disorders are divided into 1) Isolated remethylation disorders in which only tHcy concentration becomes elevated and are caused by defects in methionine synthase reductase (MSR) (complementation group cblE), methionine synthase (MS) (complementation group cblG), 5, 10-methylenetetrahydrofolate reductase MTHFR, and complementation group cblD-Hcy [4], MTHFD1 deficiency; and 2) Combined re-methylation disorders which is a heterogeneous group of diseases caused by defects in the synthesis of methylcobalamin (MeCbl) and adenosylcobalamin (AdoCbl), leading to methylmalonic aciduria (MMA) combined with hyperhomocysteinemia (HHcys) as in (CblC, CblF, CblJ, CblD, CblX, etc) [5]. Although the patterns of clinical manifestations of remethylation disorders vary with age, neurological features are prominent in both MTHFR deficiency and disorders of cobalamin metabolism. Brain MRI abnormalities are common in re-methylation defects, including diffuse cerebral atrophy, white matter changes, basal ganglia lesions, and hydrocephalus. The pathophysiological mechanisms underlying the white matter abnormalities detected by MRI have been associated with oedema and abnormal myelination. Early recognition of the re-methylation disorders, followed by aggressive treatment, may lead to a favourable clinical and biochemical outcome [6]. The current study highlights the clinical, biochemical, and genetic profile of patients with re-methylation disorders.

2. Patients and methods

A cohort study was conducted in the metabolic clinic, Children Welfare Teaching Hospital /Baghdad. Seventy-two patients presented to the clinic since 2017 with variable clinical manifestations showing hyperhomocysteinemia, out of whom fifteen patients were included in the present study because they fulfilled the following criteria: (1) elevated serum tHcy levels (>15 μmol/L); (2) low or normal blood Methionine (Met (12–40 umol/l)). Data were gathered between December 1st, 2021 and December 1st, 2022, from patients who had visited the clinic over five years. This included demographic data, clinical manifestations, family history, and biochemical findings such as tHcy, Met, B12, Folate, Hb, MCV, and brain MRI. Additionally, the patients underwent genetic testing. Thorough neurological examinations were conducted on each patient by a pediatric neurologist. A qualified radiologist reviewed magnetic resonance imaging (MRI) of the brain. All patients were tested with organic acidaemia panel sequencing. The genes responsible for these metabolic defects were incorporated into this panel. Genetic advice was obtained by discussing each case with a geneticist. tHcy was measured instead of free homocysteine (fHcy) in a private lab in Baghdad with an average value range of 5–15 mmol/l. In normal plasma, most Hcy is protein-bound and free HCy disulphide (fHCy) concentrations are negligible. Whole blood was collected into gel and clot activator tube. No special precautions around fasting or dietary protein intake were needed before sampling. However, prandial status and recent protein intake may account for the small non-clinically significant daily fluctuations in tHCy concentration observed in previous studies [2]. Plasma homocysteine was quantified by using TOSOH apparatus (model AIA-360) and ST AIA-PACK Homocysteine Kit with pretreatment set. Methionine level was measured by a High-Performance Liquid Chromatography HPLC system consisting of quaternary pumps (model YL 9110), a UV-VIS detector (model YL 9120), and a vacuum degasser (model YL 9101). All injections were done with a manual sampling injector (Model 7725i, 20 μl loop) and reversed-phase chromatography C18 columns (Agilent-AAA, 150 × 4.6 mm, particle size 3.5 μm). Levels of amino acids are relatively constant in the blood, and ideally, fasting samples are preferred for diagnosing defects in amino acid metabolism. Molecular analysis was conducted in Invitae lab in San Francisco, USA, using an organic academia panel, which includes 101 genes (supplement) and was performed in 13 patients. The test involves sequence analysis and testing deletion/duplication variants of the genes using a next-generation sequencing (NGS) technique. According to the laboratory guidelines, Genomic DNA obtained from the submitted sample is enriched for targeted regions using a hybridization-based protocol and sequenced using Illumina technology. Unless otherwise indicated, all targeted regions are sequenced with ≥50× depth or are supplemented with additional analysis. If a CNV is identified, MLPA or MLPA-sequencing is run to confirm the variant. Two samples were tested using a whole exome sequencing test at the CeGat lab in Germany as per the families' request. The thermolabile *MTHFR* polymorphisms were not included in this study. Written consent was obtained from all the patient's caregivers. Approval was obtained from the local ethical approval committee in the Children's Welfare Teaching Hospital. IBM SPSS v26 was used for statistical analysis.

3. Result

While 15 patients met the inclusion criteria, statistical analyses were performed on 14 MTHFR patients, and the single CblC patient was evaluated separately.

Table 1 shows the demographic and clinical features of the studied patients. The total number of MTHFR patients was 14, including nine females, with a female: male ratio of 1.8:1. The mean age at diagnosis was 7.1 years±4.5 SD with a range of 1–16 years. The mean age at the time of inclusion in the study was 9 years ±4.4 SD with a range of 2–16 years. Consanguineous marriage was positive in all patients except one (patient no.15). Family history of a similar disorder was recorded in 11 patients (73 %). Our cohort patients' relatedness (siblings) were as follows: P2 and P3, P5 and P6, P10 and P11, and P12 and P13, as denoted in Tables 1 and 2.

Patients presented with variable neurological manifestations. The two main reported clinical manifestations were gait disturbance (10/14, 71.4 %) and cognitive impairment/intellectual disability (6/14, 42.8 %). Ophthalmological assessment was performed in all patients, and it was unremarkable. Table 2 demonstrates the studied patients' biochemical, neuroimaging, and molecular findings. Plasma tHcy level ranged from 22 to 352 μmol/l, with a mean of 167.7 ± 91.0, and twelve patients were reported to have levels >100 μmol/l. The level of Methionine ranged from 17 to 38 μmol/l, which was within the normal range.

Vitamin B12 was tested in 10 patients, 3 of whom had their vitamin level below the lower reference range (patients no. 3, 6, and 9). The mean level was 1155.7 (50–2000 pg/ml). Serum folate was tested in 5 patients; the mean level was 8.2 ng/ml, ranging from 1.6 to 12 ng/ml. The MCV of 10 patients was available and ranged from 77 to 100 fl. The mean Hb level of ten patients was 11.9 g/dl and ranged from 9.4 to 13 g/dl. Leukodystrophy was the most frequent neuroimaging occurrence (8/14, 57.1 %). Molecular Variants were detected in *MTHFR* in 14 patients, while variant changes were observed in *MMACHC* in a single patient (c.394C > T). Among those with molecular changes in *MTHFR*, five patients shared the same homozygous variant c.1129C > T (p.Arg377-Cys) in exon 7, two patients had homozygous variant c.1262G > C (p.Trp421Ser) in exon 8, two patients with homozygous variant c.459C > G (p.Ile153Met) in exon 3, four patients had compound heterozygous variants and one patient had homozygous variant c.1223 T > G (p.

Table 1
Demographic and clinical data of 15 patients presented re-methylation disorder of HCU.

Patient no.	1#	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Demographics															
Sex	f	f	f	f	f	f	f	m	f	m	m	m	f	f	m
Family history		+	+	+	+	+	+			+	+	+	+	+	
Consanguinity	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Age of inclusion in the study (year)	10	12	13	13	14	5	2	14	9	6	5	16	9	5	2
Age of diagnosis (year)	5	6	7	13	14	5	2	11	5	5	4	16	9	1	2
Clinical manifestations															
Cognitive impairment/decreased school performance	+		+	+	+			+				+	+		
Behavioural disturbance		+		+									+	+	
Seizure	+								+	+	+				+
Gait Abnormality	+	+	+	+	+			+	+	+	+			+	+
Psychomotor delay					+		+			+	+	+			
Marfanoid habitus												+			
Microcephaly							+			+					
Abnormal pigmentation of skin and hair							+								

The patients' relatedness (siblings) were as follows: P2 and P3, P5 and P6, P10 and P11, and P12 and P13.
the CblC patient was included in the table but assessed independetly.
+ present

Leu408Arg) in exon 8 which was variant of unknown significance (VUS), as seen in Fig. 1.

4. Discussion

Management of patients with inborn errors of metabolism in Iraq was possible in 2009. An academic fellowship in metabolic specialization was established in 2021. Since then, the clinic has been registering patients with homocystinuria, of which re-methylation disorder has been identified in 20 % of individuals. To our knowledge, this is the first local study to characterise this type of disorder.

The re-methylation group of homocystinuria is a rare disorder. It was studied by Padmanabha et al. 2021 (three patients with MTHFR and the others have no available genetic tests), Chang KJ et al. 2020 (two with MTHFR and five with MMACHC-related disorders), Wei Y et al. 2019 (eight patients with MMACHC disorders), Lossos A et al. 2014 (four patients with MTHFR disorders), Bathgate D et al. 2012 and Perna A et al. 2018 (two patients each with MTHFR disorders) and Lin N et al. 2016 (one patient with MTHFR disorder). The number of patients that were enrolled in each of the aforementioned studies was smaller than the present study (15 patients) [7–13].

In the current study, female dominance in MTHFR patients is in line with the result of Lossos A. et al. (2014). However, it contrasted with the male predominance in Padmanabha et al. (5:2), and Change et al. (100 %) [7,8,10].

The age of onset and diagnosis in the current study is found to be much lower than that reported in studies of Padmanabha et al. 2021 (11–18 years, 15.6 ± 2.4 years), [7] Chang et al., 2021 (two MTHFR patients (they are thirty and fifty years old, respectively.)) and others (Lossos A et al.). The higher age ranges and mean ages reported in the studies mentioned above resulted from including adult patients in these studies, as they were enrolled in the adult neurology departments. The findings of this study should inform paediatricians, pediatric neurologists, and metabolic specialists about the significantly younger age at which this metabolic disorder presents. About one-third of MTHFR patients (14/44) have late-onset presentation, according to the *E*-HOD registry (2019) [14]. In contrast, nearly all MTHFR patients in the current study had late onset (13/14). The variation in sample size and the relation to the underlying molecular profile that was not listed in the E-HOD registry may explain this discrepancy.

A high rate of consanguineous marriages is reported in the current study, which was similar to that identified by N. Aljassim et al. in Saudia Arabia (five patients out of seven reported positive consanguinity) [15]. The high consanguinity rate among first/s-degree cousins in an Arab population in general and Iraqi society in particular [16], may explain the higher rate of consanguinity reported in the current study compared to studies from Far East countries.

Gait disturbance was the predominant clinical manifestation in MTHFR patients, which was reported in 10 patients, among whom one manifested it as a main presenting feature. Other studies (Padmanabha et al., and Chang et al.) reported gait abnormality (spastic paraparesis) as the main presenting feature. Those studies involved older children and adults [7,8].

Other clinical manifestations that were evidenced in the studied MTHFR patients during their course of illness were: 1) Cognitive impairment in 6/14 (42.9 %) patients, which was initially recognised as a decline in school performance, a result that is similar to other studies [7,8]. Out of those six patients, two manifested cognitive impairment as part of psychomotor delay (see item four); 2) seizure in 4/14 (28.6 %) patients (their identified variant (c.680C > T) was also found in 3 patients in N. Aljassim et al). Seizures has been documented in other studies as well [6,7,8,15]; 3) Behavioural abnormalities like autistic behaviour and hyperactivity were presented in nearly one-fourth (4/14) of the cohort. Padmanabha et al. noted that more patients had this manifestation (two genetically confirmed MTHFR patients) [7,8]; 4) Psychomotor delay (where delay in cognitive/motor skills have been manifested since very early months of life and thus the children weren't reported to be healthy) was noted in 5 patients (35.7 %); 5)Two patients with MTHFR variants showed microcephaly. N Aljassim et al. [15] found two patients with microcephaly to have homozygous c.680C > T, while this study reports this variant in a compound heterozygous state; 5) although marfanoid habitus is not a unique feature in non-classical HCU, it has been observed in this study (patient no. 12 (Fig. 2)) and Padmanabha et al.'s study (2 patients). Therefore, non-classical HCU need to be considered among the differential diagnoses when confronting such manifestation; and 6) Fair hair was manifested in patient no.7. Padmanabha et al. likewise reported one patient with hypopigmented hair and marfanoid habitus who was deficient in MTHFR [7].

Concerning the course of illness, neuro-regression (loss of cognitive function, personality changes, vision loss, hearing loss, tone abnormalities, and epilepsy) was reported in six patients (42.9 %).

Biochemically, 12 (80 %) patients of MTHFR had severe hyperhomocysteinemia (> 100 μmol /L). One patient (no. 2) had a moderate elevation (44 mmol). While patient no.13 had mildly elevated tHcy (22 μmol/l) [17]. The latter patient is a girl who was confirmed genetically to have a c.459C > G variant in *MTHFR*. After her older brother's genetic diagnosis, she underwent testing and was diagnosed. The investigation of both siblings with HPLC of amino acids (double-checked in fasting

Table 2
Biochemical, radiological, and genetic profile of 15 patients with re-methylation disorder of HCU.

Patient no.	tHcy (5-15 μmol/l)[d]	Met (12–40 μmol/l)[d]	B12 (200–800 pg/ml)	Folate (3-17 ng/ml)[d]	Hb (10.5–13.5 g/dl)[d]	MCV fl[d]	Radiological findings	Genetic analysis	Treatment modalities And outcome
1[a]	75	30	200	NA	13	96	Brain atrophy with microangiopathic changes and basal ganglia symmetrical involvement	*MMACHC* Exon 3 c.394C > T (p. Arg132 Ter) P Homozygous	B12[c], Betaine and L- L-carnitine Marked improvement of gait and intellectual functions
2	44	36	500	NA	NA	NA	Normal	*MTHFR* Exon 8 c.1262G > C(p. Trp421Ser) P Homozygous	B12[c], Betaine and folinic acid No clinical improvement (poor compliance)
3	245	38	197	NA	12	81	Leukodystrophy	*MTHFR* Exon 8 c.1262G > C(p. Trp421Ser) P Homozygous	B12[c], Betaine and folinic acid No clinical improvement (poor compliance)
4	314	31	769	4.7	13	86	Brain atrophy	*MTHFR* Exon 7 c.1129C > T(p. Arg377Cys) P Homozygous	B12[c], Betaine and folinic acid Marked improvement of motor function (became ambulatory) and behavioural disturbance
5	352	30	NA	NA	11	82	Brain atrophy with leukodystrophy	*MTHFR* Exon 7 c.1129C > T(p. Arg377Cys) P Homozygous	B12[c], Betaine and folinic acid Marked improvement of motor function (became ambulatory) and behavioural disturbance
6	149	31	50	NA	11	77	Normal	*MTHFR* Exon 7 c.1129C > T (p. Arg377Cys) P Homozygous	B12[c], Betaine and folinic acid Improvement of intellectual function and speech
7	192	22	NA	NA	9.4	80	Normal	*MTHFR* Exon 7 c.1129C > T (p. Arg377Cys) P Homozygous	B12[c], Betaine and folinic acid Loss of follow-up
8	110	19	NA	NA	12	84	Leukodystrophy With angiopathic changes	*MTHFR* Exon 7, Exon 3 c.1129C > T (p. Arg377Cys) P c.470G > A(p. Arg157Gln) P Compound Heterozygous	B12[c], Betaine and folinic acid Improvement of gait
9	139	22	192	1.6	13	100	Leukodystrophy with angiopathic changes	*MTHFR* Exon 8 c.1223 T > G(p. Leu408Arg) VUS Homozygous	B12[c], Betaine and folinic acid Died
10	170	36	2000	NA	12	85	Leukodystrophy with cerebral atrophy	*MTHFR* Exon 5, Exon 7 c.680C > T(p. Thr227Met)P c.1129C > T(p. Arg377Cys) P Compound Heterozygous	B12[c], Betaine and folinic acid Mild improvement in gait
11	121	37	2000	12	NA	NA	Leukodystrophy	*MTHFR* Exon 5, Exon 7 c.680C > T(p. Thr227Met) P c.1129C > T(p. Arg377Cys) P Compound Heterozygous	B12[c], Betaine and folinic acid Mild improvement in gait

(*continued on next page*)

Table 2 (*continued*)

Patient no.	tHcy (5-15 μmol/l)[d]	Met (12–40 μmol/l)[d]	B12 (200–800 pg/ml)	Folate (3-17 ng/ml)[d]	Hb (10.5–13.5 g/dl)[d]	MCV fl[d]	Radiological findings	Genetic analysis	Treatment modalities And outcome
12[b]	130	17	2000	12	13	85	Normal	*MTHFR* Exon 3 c.459C > G(p. Ile153Met) LP Homozygous	B12[c], Betaine and folinic acid No clinical improvement
13 [b]	22	20	NA	NA	13	82	Normal	*MTHFR* Exon 3 c.459C > G(p. Ile153Met) LP Homozygous	B12[c], Betaine No clinical improvement
14	159	24	578	NA	NA	NA	Leukodystrophy with angiopathic changes	*MTHFR* Exon 7 c.1129C > T(p. Arg377Cys) P Homozygous	B12[c], Betaine and folinic acid No clinical improvement
15	201	37	960	10.8	NA	NA	Leukodystrophy	*MTHFR* Exon 5, Exon 7 c.680C > T(p. Thr227Met) P c.1129C > T (p. Arg377Cys) P Compound Heterozygous	B12[c], Betaine and folinic acid Loss of follow-up

Abbreviation: Hb = haemoglobin, **MCV** (mean corpuscular volume) [1 year (70–82) 2–6 year (72–87) 6–12 year (76–90) 12–18 Female (77–94) Male (77–920)], **NA** = not available P = pathogenic, LP = likely pathogenic.

[a] The patient with CblC was assessed independently. The patients' relatedness (siblings) were: P2 and P3, P5 and P6, P10 and P11, and P12 and P13.

[b] The investigations of both siblings with HPLC of amino acids (double-checked in fasting status) revealed an elevation of phenylalanine (< 600 μmol/L). Therefore, hyperphenylalaninemia was discovered coincidentally.

[c] Intramuscular Hydroxycobalamen.

[d] These laboratory values are before treatment.

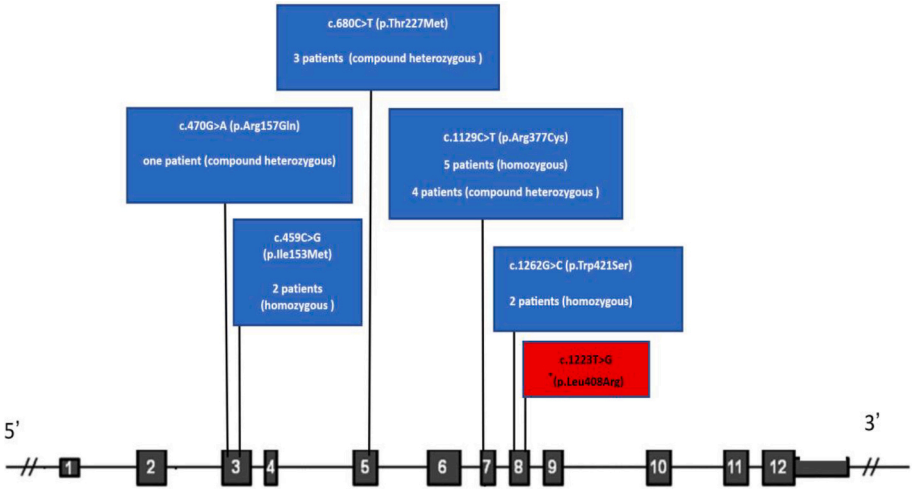

Fig. 1. Schematic representation of the location and frequency of the variants detected on the *MTHFR* in the studied patients.
*(red coloured flag) = the variant detected is VUS

status) revealed an elevation of phenylalanine (< 600 μmol/L). Therefore, hyperphenylalaninemia was discovered coincidentally. It was a difficult situation because a low-protein diet for hyperphenylalaninemia will lower methionine levels in the blood. They were, therefore, closely monitored for methionine during their diet. The parents could not afford genetic confirmation of hyperphenylalaninemia and parental consanguinity can be a key point in explaining the presence of dual genetic disorders in children. According to Padmanabha et al., the frequency of severe hyperhomocysteinemia was comparable to that of our group (86 %) [7]. The severity of tHcy was not related to the severity of the disease, as previously reported [8,14]. Serum B12 level was low in three patients with MTHFR. Even after giving them vitamin B12 supplements, two had consistently high serum homocysteine levels despite normalization of serum B12 level. We lost contact with the third patient with VUS in *MTHFR*. It would appear from this that dietary deficiencies were not the cause of the patient's elevated homocysteine.

White matter lesions were reported in two forms: 1) eight (53 %) patients showed deep and periventricular white matter symmetrical distribution, with a posterior predominance (four patients). Which can be listed under the general term genetic leukoencephalopathy. Padmanabha et al. and Chang et al. described such a white matter involvement in 72 % and 29 %, respectively; 2) Microangiopathic changes, manifested as foci of signal abnormalities in the deep white matter area (Fazekas I and II) [18], were identified in 3/14 (21.4 %). One patient

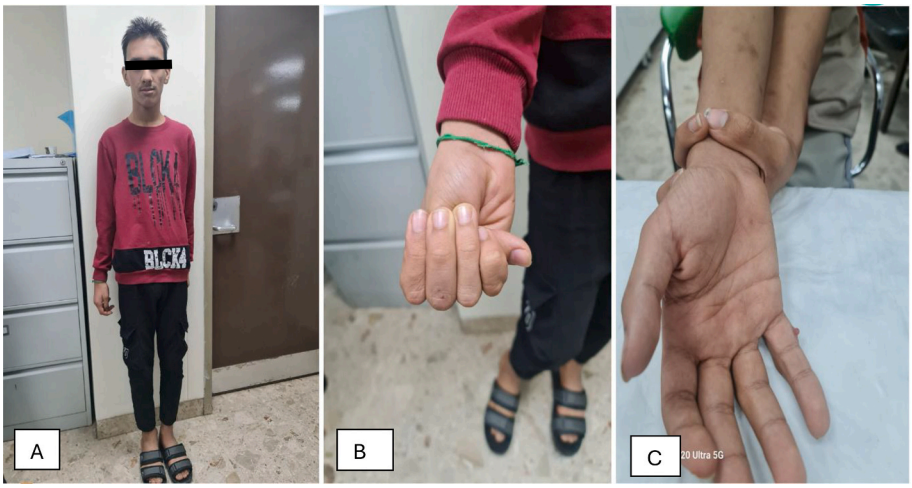

Fig. 2. (patient no.12): (A) 16-year-old male presented with intellectual disability and marfanoid habitus (B) thumb sign (C) wrist sign.

had brain atrophy, as demonstrated by Padmanabha et al. and N. Aljassim et al. studies [7,15]. At the same time, combined findings were seen in 5/14 (35.7 %), as seen in Fig. 3.

The current study showed that almost all patients with re-methylation disorder showed mutations in *MTHFR*. The same finding was observed by Padmanabha et al. It did not, however, agree with Chang et al., Wei et al. and others who found Cb1C to be the commonest cause of re-methylation disorder. [6–9, 14,19.] Whereas the *MTHFR* mutation was discovered to be the second most frequent cause of re-methylation disorder, after the CblC defect. [6,14,19]

The commonest variant that was reported in *MTHFR* was c.1129C > T (9, 64 %), followed by c.459C > G (2, 14.3 %), which was identified by Padmanabha et al. Two patients had a c.680C > T variant in a heterozygous status, as reported in the study of N. Aljassim et al. (7 patients). Our study group may share the latter mutation with the Saudian population (a founder mutation in Saudi Arabia) due to the displacement of tribes between different Arabian regions [7,15]. A novel mutation (c.1223 T > G) was identified in one patient (no. 9) and classified as VUS. Although in silico analysis was not available to confirm the pathogenicity of this variant, its compatibility with the clinical and

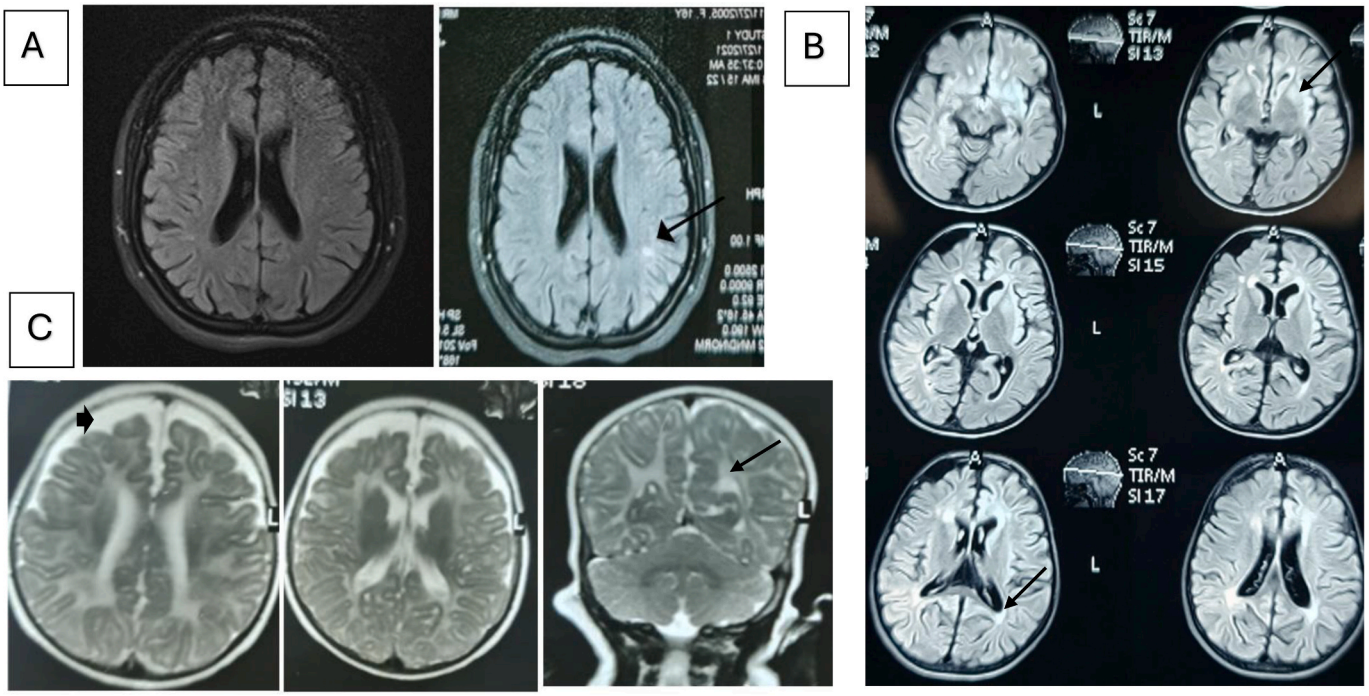

Fig. 3. (A) Patient no. 5: A 15 - year - old female, suffered from psychomotor delay and abnormal behaviour which led to her condition progressing and eventually ending in a nonambulatory state. Brain MRI (axial/FLAIR) shows multiple asymmetrical subcortical white matter lesions with high signal intensities in the posterior region associated with generalised mild brain atrophy and dilated lateral ventricle. She had confirmed genetic variant in *MTHFR*. (B) Patient no. 1: A 10-year-old girl presented with neuro-regression, decreased school performance and seizure. Brain MRI (axial / FLAIR) shows high signal abnormalities in the periventricular and deep white matter (anteriorly and posteriorly), basal ganglia (caudate and putamen) (black arrows on both areas), and mild and generalised brain atrophy. She had a confirmed genetic variant in *MMACHC*. (C) Patient no.14: A 5 – year – old female, presented with neuro-regression started at one year, abnormal gait, and abnormal behaviour. Brain MRI (axial and coronal T2WI) shows bilateral symmetrical diffused increased signals of white matter (black arrow) (periventricular, deep and subcortical) with decreased white matter volume and atrophic changes that are more prominent at the frontal areas (black arrow head). He had a confirmed genetic variant in *MTHFR*.

biochemical phenotype of re-methylation disorder, and its segregation in her parents increases the possibility that it is a disease-causing variant. The child manifested severe neurological features.

The variant c.1129C > T was found in 5 patients, from four unrelated families. Three families were from the same province (Dhiyala, which is located in the far east of Iraq), which may suggest that this mutation is a founder one. We couldn't get more information about their tribal details.

All patients were placed on treatment before obtaining the genetic confirmation of the disorder. These included folinic acid, intramuscular vitamin B12 (hydroxocobalamin), oral L-carnitine, and oral Betaine. After obtaining the genetic results, the management was adjusted according to the guidelines [6].

Three out of fourteen patients showed significant clinical improvement. Two MTHFR patients began walking after being wheelchair dependent, one MTHFR patient demonstrated improved speech and intellectual abilities. Three additional MTHFR patients exhibited a slight improvement in gait.

The patient (no.1) with CblC disease was born to parents who were consanguineous. She was five years old when she first showed signs of cognitive impairment, and later a decline in academic performance, seizures, and ataxic gait. She had moderate hyperhomocysteinemia. Her brain MRI revealed a combination abnormalities, including micro-angiopathic changes, brain atrophy, and lesions at the basal ganglia (Fig. 3 B). Regarding she harboured a variant (c.394C > T) in *MMACHC* in a homozygous state. The same variant was reported by Wei et al. in a compound heterozygous state in a 13–year–old boy. [9] This mutation is reported to be associated with late-onset presentation of Cb1C disorder [20,21].This genotype/phenotype correlation was attributed to the transcript level. The expression and influence of this allele *MMACHC* mRNA lead to late-onset mutant proteins, which are more stable than those responsible for early onset [22]. After beginning treatment, the patient's cognitive and ambulatory abilities significantly improved, allowing her to return to school.

This study, to the best of our knowledge, is the first to describe re-methylation disorder in Iraq. However, it should be noted that it has several limitations, such as a small sample size; the inclusion of all patients with re-methylation disorder regardless of the underlying genetic or metabolic aetiology, which may statistically confound the results; the inclusion of two patients who have two different molecular disorders (MTHFR disorder and PKU), which will further confound the analysis; and the Financial constraints prevented the assessment of methylmalonic acid in urine and the use of high throughput genetic tests like WES in all patients for a more precise assessment

5. Conclusion

According to this study, individuals with homocysteine remethylation disorders can manifest symptomatology such as neuroregression, psychomotor delay, and whiter matter changes younger than anticipated. Non-classical homocystinuria is a disorder that is amenable to treatment. Although a Marfanoid habitus is commonly associated with classical homocystinuria, it can also occur in the non-classical type. Genetic testing is crucial in identifying the specific mutation type and guiding definitive treatment

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CRediT authorship contribution statement

Mays R. Al-Tai: Writing – review & editing, Writing – original draft, Validation, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Adel A. Kareem:** conceptualization, writing - original draft, supervision. **Nebal W. Saadi:** Writing - review & editing, Data curation, Formal analysis. **Tawfig Ben Omran:** Formal analysis, Data curation. **Ban A. Abdul Majeed:** Data curation. **Ibrahim F. Ibrahim:** Data curation. **Lamia A. Alattar:** Formal analysis.

Declaration of competing interest

None.

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Appendix A. Appendix

GENE	TRANSCRIPT
ABCD4	NM_005050.3
ACAD8	NM_014384.2
ACADSB	NM_001609.3
ACAT1	NM_000019.3
ACSF3	NM_174917.4
ADK	NM_001123.3
AGK	NM_018238.3
AHCY	NM_000687.3
ALDH6A1	NM_005589.3
AMN*	NM_030943.3
ASPA	NM_000049.2
ATP5D	NM_001001975.1
AUH	NM_001698.2
BCKDHA	NM_000709.3
BCKDHB	NM_183050.2
BOLA3	NM_212552.2
BTD	NM_000060.3
C19orf70	NM_205767.2
CBS	NM_000071.2
CD320	NM_016579.3
CLPB	NM_030813.5
CPS1	NM_001875.4
CUBN	NM_001081.3

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GENE	TRANSCRIPT
D2HGDH	NM_152783.4
DBT	NM_001918.3
DHTKD1	NM_018706.6
DLD	NM_000108.4
DNAJC19	NM_145261.3
ECHS1	NM_004092.3
ETFA	NM_000126.3
ETFB	NM_001985.2
ETFDH	NM_004453.3
ETHE1	NM_014297.3
FBP1	NM_000507.3
FH*	NM_000143.3
FLAD1	NM_025207.4
FTCD	NM_006657.2
GCDH	NM_000159.3
GIF	NM_005142.2
GLRX5	NM_016417.2
GLYCTK	NM_145262.3
GNMT	NM_018960.5
GSS	NM_000178.2
HCFC1	NM_005334.2
HIBCH	NM_014362.3
HLCS	NM_000411.6
HMGCL	NM_000191.2
HSD17B10	NM_004493.2
HTRA2	NM_013247.4
IBA57	NM_001010867.3
IDH2	NM_002168.3
ISCA2	NM_194279.3
IVD	NM_002225.3
L2HGDH	NM_024884.2
LIAS	NM_006859.3
LIPT1	NM_145199.2
LIPT2	NM_001144869.2
LMBRD1	NM_018368.3
MAT1A	NM_000429.2
MCCC1	NM_020166.4
MCCC2	NM_022132.4
MCEE	NM_032601.3
MLYCD	NM_012213.2
MMAA	NM_172250.2
MMAB	NM_052845.3
MMACHC	NM_015506.2
MMADHC	NM_015702.2
MTHFR*	NM_005957.4
MTR	NM_000254.2
MTRR	NM_002454.2
MUT	NM_000255.3
NFU1	NM_001002755.2
OGDH	NM_002541.3
OPA3	NM_025136.3
OPLAH	NM_017570.4
OXCT1	NM_000436.3
PCCA	NM_000282.3
PCCB	NM_000532.4
PCK1	NM_002591.3
POLG	NM_002693.2
PPM1K	NM_152542.4
PRDX1	NM_002574.3
SERAC1	NM_032861.3
SLC13A3	NM_022829.5
SLC13A5	NM_177550.4
SLC25A1	NM_005984.4
SLC25A19	NM_021734.4
SLC25A32	NM_030780.4
SLC52A1	NM_017986.3
SLC52A2	NM_024531.4
SLC52A3	NM_033409.3

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GENE	TRANSCRIPT
SUCLA2	NM_003850.2
SUCLG1	NM_003849.3
SUGCT	NM_024728.2
TAZ	NM_000116.4
TCN1	NM_001062.3
TCN2	NM_000355.3
THAP11	NM_020457.2
TIMM50	NM_001001563.3
TMEM70	NM_017866.5
ZNF143	NM_003442.5

Data availability

The authors do not have permission to share data.

References

[1] A. Gerrard, C. Dawson, Homocystinuria diagnosis and management: it is not all classical, J. Clin. Pathol. 75 (2022) 744–750.

[2] Y.N. Li, S. Gulati, P.J. Baker, L.C. Brody, R. Banerjee, W.D. Kruger, Cloning, mapping and RNA analysis of the human methionine synthase gene, Hum. Mol. Genet. 5 (12) (1996) 1851–1858.

[3] S.L. Sunden, M.S. Renduchintala, E.I. Park, S.D. Miklasz, T.A. Garrow, Betaine-homocysteine methyltransferase expression in porcine and human tissues and chromosomal localization of the human gene, Arch. Biochem. Biophys. 345 (1) (1997) 171–174.

[4] Eva Richard, Sandra Brasil, Fatima Leal, et al., Isolated and combined remethylation disorders: biochemical and genetic diagnosis and pathophysiology, J. Inborn Errors Metabol. Screen. 5 (2017) 1–11.

[5] D.S. Froese, S. Healy, M. McDonald, et al., Thermolability of mutant MMACHC protein in the vitamin B12-responsive cblC disorder, Mol. Genet. Metab. 100 (1) (2010) 29–36.

[6] Martina Huemer, Daria Diodato, Bernd Schwahn, et al., Guidelines for diagnosis and management of the cobalamin-related remethylation disorders cblC, cblD, cblE, cblF, cblG, cblJ and MTHFR deficiency, J. Inherit. Metab. Dis. 40 (2017) 21–48.

[7] Hansashree Padmanabha, Rohan Mahale, Rita Christopher, et al., Clinical, biochemical, radiological, and genetic profile of patients with homocysteine Remethylation pathway defect and spastic paraplegia, Ann. Indian Acad. Neurol. 24 (6) (2021) 908–916.

[8] K.J. Chang, Z. Zhao, H.R. Shen, Q. Bing, N. Li, X. Guo, et al., Adolescent/adult-onset homocysteine remethylation disorders characterized by gait disturbance with/without psychiatric symptoms and cognitive decline: a series of seven cases, Neurol. Sci. 42 (2021) 1987–1993.

[9] Y. Wei, Y. Zhou, J. Yuan, J. Ni, M. Qian, L. Cui, et al., Treatable cause of hereditary spastic paraplegia: eight cases of combined homocysteinaemia with methylmalonic aciduria, J. Neurol. 266 (2019) 2434–2439.

[10] A. Lossos, O. Teltsh, T. Milman, V. Meiner, R. Rozen, D. Leclerc, et al., Severe methylenetetrahydrofolate reductase deficiency: clinical clues to a potentially treatable cause of adult-onset hereditary spastic paraplegia, JAMA Neurol. 71 (2014) 901–904.

[11] Deborah Bathgate, Patrick Yu-Wai-Man, Brian Webb, Robert W. Taylor, et al., Recessive spastic paraparesis associated with complex I deficiency due to MTHFR mutations, J. Neurol. Neurosurg. Psychiatry 83 (2012) 115.

[12] A. Perna, M. Masciullo, A. Modoni, E. Cellini, E. Parrini, E. Ricci, et al., Severe 5,10-methylenetetrahydrofolate reductase deficiency: a rare, treatable cause of complicated hereditary spastic paraplegia, Eur. J. Neurol. 25 (2018) 602–605.

[13] N. Lin, N. Jiang, Y. Dai, J. Gao, L. Wang, Adult-onset severe methylenetetrahydrofolate reductase deficiency characterized by reversible spastic paraplegia with a novel mutation, Neurol. Sci. 37 (2016) 1735–1737.

[14] Martina Huemer, Daria Diodato, Diego Martinelli, et al., Phenotype, treatment practice and outcome in the cobalamin- dependent remethylation disorders and MTHFR deficiency: data from the E-HOD registry, J. Inherit. Metab. Dis. 1–20 (2019).

[15] Nada Aljassim, Majid Alfadhel, Marwan Nashabat, Wafa Eyaid, Clinical presentation of seven patients with methylenetetrahydrofolate reductase deficiency, Mol. Genet. Metabol. Reports 25 (2020) 100644.

[16] Ghazi O. Tadmouri, Pratibha Nair, Tasneem Obeid, et al., Consanguinity and reproductive health among Arabs, Reprod. Health 6 (2009) 17.

[17] Fuad Al Mutairi, Hyperhomocysteinemia: clinical insights, J. Central Nervous Syst. Disease 12 (2020) 1–8.

[18] P. Sclaeltens, F. Barkhof, D. Leys, et al., A semiquantative rating scale for the assessment of signal hyperintensities on magnetic resonance imaging, J. Neurol. Sci. 114 (1993) 7–12.

[19] L. Dean, Methylenetetrahydrofolate reductase deficiency, in: V.M. Pratt, S.A. Scott, M. Pirmohamed, et al. (Eds.), Medical Genetics Summaries [Internet], National Center for Biotechnology Information (US), Bethesda (MD), 2012.

[20] W. Nyhan, G. Hoffmann, Atlas of Inherited Metabolic Diseases, Fourth edition, Taylor & Francis Group, LLC, 2020.

[21] J.P. Lerner-Ellis, N. Anastasio, J. Liu, et al., Spectrum of mutations in MMACHC, allelic expression, and evidence for genotype-phenotype correlations, Hum. Mutat. 30 (7) (2009) 1072–1081, https://doi.org/10.1002/humu.21001.

[22] Xianling Wang, Yanhui Yang, Xuying Li, et al., Distinct clinical, neuroimaging and genetic profiles of late-onset cobalamin C defects (cb1C): a report of 16 Chinese cases, Orphanet J. Rare Dis. 14 (2019) 109.