Clinical pattern, mutations and in vitro residual activity in 33 patients with severe 5, 10 methylenetetrahydrofolate reductase (MTHFR) deficiency

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Abstract

Background Severe methylenetetrahydrofolate reductase (MTHFR) deficiency is a rare inborn defect disturbing the remethylation of homocysteine to methionine (<200 reported cases). This retrospective study evaluates clinical, biochemical genetic and in vitro enzymatic data in a cohort of 33 patients.

Methods Clinical, biochemical and treatment data was obtained from physicians by using a questionnaire. MTHFR activity was measured in primary fibroblasts; genomic DNA was extracted from cultured fibroblasts.

Results Thirty-three patients (mean age at follow-up 11.4 years; four deceased; median age at first presentation 5 weeks; 17 females) were included. Patients with very low (<1.5 %) mean control values of enzyme activity (n=14) presented earlier and with a pattern of feeding problems, encephalopathy, muscular hypotonia, neurocognitive impairment, apnoea, hydrocephalus, microcephaly and epilepsy. Patients with higher (>1.7–34.8 %) residual enzyme activity had mainly psychiatric symptoms, mental retardation, myelopathy, ataxia and spasticity. Treatment with various combinations of betaine, methionine, folate and cobalamin improved the biochemical and clinical phenotype. During the disease course, patients with very low enzyme activity showed a progression of feeding problems, neurological symptoms, mental retardation, and psychiatric disease while in patients with higher residual enzyme activity, myelopathy, ataxia and spasticity increased. All other symptoms remained stable or improved in both groups upon treatment as did brain imaging in some cases. No clear genotype-phenotype correlation was obvious.

Discussion MTHFR deficiency is a severe disease primarily affecting the central nervous system. Age at presentation and clinical pattern are correlated with residual enzyme activity. Treatment alleviates biochemical abnormalities and clinical symptoms partially.

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Introduction

Severe methylenetetrahydrofolate reductase (MTHFR) deficiency [MIM 607093; *MTHFR*; 1p36.22] is a rare inborn error of metabolism inherited in an autosomal recessive manner. The enzyme MTHFR catalyses the reduction of 5,10 methylenetetrahydrofolate to 5-methyltetrahydrofolate, which is the most

common form of folate in blood, cerebrospinal fluid and tissues and serves as a methyl donor for the methylation of homocysteine (Hcy) to methionine (Met). In MTHFR deficiency, methylation of Hcy to Met is decreased resulting in highly elevated plasma total Hcy (tHcy) and low plasma Met concentrations (Fattal-Valevski et al 2000; Thomas and Rosenblatt 2005; Burda et al 2015). Low Met results in a depletion of S-adenosylmethionine, which is the main donor for many methylation reactions including the synthesis of creatine, and RNA and DNA methylation (Thomas and Rosenblatt 2005). Elevated tHcy is associated with thromboembolic events and neurodevelopmental disturbances (Naughten et al 1998).

Reports on the long-term course and outcome in patients with severe MTHFR deficiency are scarce and have not been assembled systematically; present knowledge on the natural course of the disease is predominantly derived from case reports or small case series reporting in total on less than 200 individuals. Patients with severe MTHFR deficiency typically present in the neonatal period with feeding problems, failure to thrive, muscular hypotonia, microcephaly, encephalopathy and seizures. However, late-onset forms of the disease with a more variable picture encompassing delayed developmental milestones, cognitive impairment and/or gait abnormalities, psychiatric disorders or thromboembolic events have also been reported (Goyette et al 1995; Thomas and Rosenblatt 2005; Schiff et al 2011; D'Aco et al 2014; Lossos et al 2014). MRI imaging of the brain often reveals white matter disease and brain atrophy (Thomas and Rosenblatt 2005; Michot et al 2008).

Single reports have described a benefit of treatment with folinic acid (Crushell et al 2012) or methionine supplementation (Abeling et al 1999); however, the mainstay of treatment is betaine (Strauss et al 2007). In a meta-analysis of 36 patients from 15 reports, it has recently been shown that early treatment with 100 mg/kg/day of betaine prevented mortality and resulted in normal psychomotor development in five patients. With delayed treatment psychomotor development stabilised or improved, but was not restored to normal (Diekman et al 2014).

Since most of the mutations causing severe MTHFR deficiency are private (Tonetti et al 2003; Burda et al 2015) and significant intrafamiliar variation of the clinical phenotype has been described (Haworth et al 1993), no clear genotype-phenotype correlations have been outlined. A relation between in vitro enzyme activity and clinical course has occasionally been postulated (Goyette et al 1995; Birnbaum et al 2008; Forges et al 2010) but has not yet been systematically investigated.

The aims of this study are to document clinical symptoms at presentation, symptoms developing during the course of the disease, biochemical parameters and outcome in a cohort of 33 patients with MTHFR deficiency. Treatment strategies are described and possible correlations between genetic and enzymatic data and clinical findings investigated.

Methods

The local ethics committee (KEK-ZH-No. 2013–0012) approved this retrospective study. A questionnaire (provided in detail as Supplementary material) addressing physicians was constructed to obtain information on gender, age at diagnosis, problems during pregnancy and the perinatal period, clinical symptoms and metabolic parameters at initial workup and during follow-up, as well as treatment in patients with severe MTHFR deficiency. The questions were based on signs and symptoms reported in the literature on the disease. Open questions left space to report new or unexpected disease characteristics. The World Health Organization (WHO) percentiles were used to evaluate birth weight and head circumference. All physicians who sent cultured fibroblasts to Switzerland for diagnostic purposes in which severe MTHFR deficiency had been proven (76 cell lines) were asked to complete the survey after obtaining informed consent from their patient(s) and /or the caregivers. DNA was extracted and sequenced from primary fibroblasts derived from skin biopsies obtained for diagnostic purposes. MTHFR activity was measured using the physiological forward assay as described earlier, which allows reliable detection of residual activities and measurement of kinetic parameters (Suormala et al 2002) with minor modifications (Burda et al 2015). In cell lines with very low MTHFR activity (<1.5 % mean control value) only enzymatic activity and FAD-responsiveness were measured. For those with higher residual activity (>1.5 % mean control value), further enzymatic characterization (FAD responsiveness, km for NADPH) was performed (Burda et al 2015).

Results

Socio-demographic data

Clinical, biochemical, enzymatic and genetic data were available for 33 unrelated patients with MTHFR deficiency (17 females, 16 males; born between 1977 and 2013). Data on molecular genetic analysis of 76 MTHFR deficient patients, followed by extensive enzymatic characterisation from 72 of these have been published by Burda et al 2015. The group of patients included in the present study is a subgroup of this cohort. No clinical information could be obtained for 43 patients. Nine physicians did not follow the invitation to participate in the study. For the remaining patients, physicians presently involved in patient care could not be retrieved or no contact to patients or families could be established.

Consanguinity was present in 13 families; in seven families history suggested more than one individual to be affected. Eight patients were of Turkish, seven of middle-European (Austria, France, Germany, Switzerland), five of Pakistani,

five of South European (Spain, Italy), three of East European (Poland, Russia) and two of North European (UK) decent; two patients originated from the Near East (Israel, Syria) and one from Japan. All patients had been identified by selective, symptom orientated metabolic workup. Twenty-nine patients were alive at the time of data collection (mean age at follow up 11.4 years; median 9.6 years; range 4 months to 37.7 years; mean time of follow-up 9.2 years; median 8.8 years; range 3.8 months to 22.8 years). Four patients had died at ages 5, 6, 11 and 20 months, due to apnoea (n=2), apnoea in the circumstance of thrombosis of the pulmonary artery (n=1) or infection (n=1).

Pregnancy and delivery

Non-specific adverse events during pregnancy were reported in five of 33 cases (one intrauterine growth retardation, two episodes of maternal bleeding and two cases of maternal diabetes mellitus). Mean gestational week at delivery was 39.5 weeks (median 40, range 36 to 41 weeks). Birth weight was within the normal ranges (mean 3285 g; median 3383, range 2270 to 4100 g) in all but two patients who had a birth weight below the 3rd percentile. Mean percentile of head circumference at birth was the 34th (median 25th; range 2ndto 90th). Mean APGAR scores were 8.7 at minute one and 9.7 at minute ten; no major perinatal problems were reported.

Age at onset and time to diagnosis

Information on age at first disease symptoms was available for 30 patients. Median age at onset of symptoms was 1.25 months (mean 21; range 0.1 to 216 months). In 14 patients, first symptoms were observed within the first month of life; in another 11 patients by the 6 months of life. The remaining five patients became symptomatic at the ages of 2, 5, 11, 13 and 18 years. Patients with very low residual MTHFR activity (<1.5 %; n=14) presented earlier in life (mean 1.5 months, median 1 month; range 0.1 to 6 months) than those with MTHFR activity between 1.7 and 34.8 % (n=19; mean 36 months, median 2.5 months; range 0.1 months to 216 months).

Information on age at diagnosis was additionally available for 29 patients. Mean time to diagnosis was 16.6 months (median 2.8; range 0 to 108 months). Time between first symptoms and establishment of diagnosis correlated with age at first symptoms (r=0.82) and was shorter in patients with early compared to later onset of first disease symptoms.

Clinical presentation and course

For the total cohort of 33 patients, main presenting clinical symptoms were muscular hypotonia, feeding problems/failure to thrive, developmental delay/mental retardation, microcephaly and signs of encephalopathy (including lethargy and confusion). While in particular encephalopathy, lethargy and muscular hypotonia and to a lesser extent apnoea, feeding problems/failure to thrive, confusion, microcephaly and epilepsy improved, mental retardation and neurological symptoms such as abnormal gait and spasticity became more frequent over time (Fig. 1).

Open questions for "other abnormalities" revealed reports of nystagmus in three patients with very low enzyme activity and in a single patient with 3.1 % of residual enzyme activity. Optic nerve atrophy was observed in two patients with 3.8 and 10.4 % residual activity. Transient macrocytosis or macrocytic anaemia was noticed in four patients with very low enzyme activity. Isolated normo- or microcytic anaemia (n=6) and anaemia with low platelets and leukocytes (n=2) were equally distributed between the subgroups. Venous thrombosis was observed in a single case.

Brain imaging reports

Brain MRI reports from local radiologists were available for 28 patients; main symptoms are summarized in Table 2. MRI scans were considered normal in three patients (residual enzyme activities<1.5; 6.3 and 17 %). The most frequently reported pathologies were brain atrophy in 15 patients (n=7 enzyme activity<1.5 %, n=8 with residual activities between 1.8 and 9.9 %) and white matter disease/delayed myelination in 12 patients (n=3 enzyme activity <1.5%; n=9 with enzyme activities between 1.7 and 34.8 %). Enlarged ventricles/hydrocephalus were present in eight patients (n=5 enzyme activity <1.5%; n=3 patients with enzyme activity between 1.8 and 3.1 %). Interestingly, in some cases MRI images showed stabilisation or even improvement of brain pathology over time. In one child with cerebral atrophy, hypomyelination and enlarged ventricles at age 3 months, the follow-up MRI at age 3 years was considered normal (enzyme activity<1.5 %). In another patient, white matter hyperintensities at day 10 had normalized by the age of 1.5 months and remained normal until follow-up at the age of 28 months (enzyme activity 1.7 %). Treatment resulted in dramatic improvement of periventricular signal alterations within 8 months in another patient with residual enzyme activity of 3 % and in three patients, leukoencephalopathy remained stable over years under treatment (enzyme activity between 2.0 and 9.7 %). No relation between brain imaging results and enzyme activity was evident.

Biochemical parameters

As expected, plasma tHcy, plasma free homocystine and urine homocystine concentrations were higher and Met concentrations were lower at diagnosis/before treatment compared to measurements at follow-up (Table 1). tHcy and Met levels at

first presentation varied widely and were not correlated with residual enzyme activity, type/location of mutation or age at first symptoms.

Outcome

Physicians were asked to name what was, in their consideration, the most burdensome symptom for their patient. Neurocognitive impairment (retardation or developmental delay, learning problems and impaired speech) was mentioned for 19 cases, neurological symptoms (seizures, peripheral neuropathy, paraparesis and spasticity) were considered the most burdensome for four cases and eye disease (poor fixation, visual impairment) for three cases.

Treatment

Treatment approaches were very heterogeneous regarding choice, combination and dosage of drugs. Thirty-one patients received betaine (dose range 200 to 24,000 mg/day). Sixteen patients were treated with folic acid; three patients received a combination of folate/folinic acid and 12 folinic acid alone (dose range 3 to 100 mg per day). Cobalamin preparations were used in 23 patients (n=19 hydroxocobalamin: dose range from 1 mg IM per month to 1 mg/day IM/ PO; n=4 cyanocobalamin: dose range from 0.5 mg every month - 1 mg/day). Twelve patients received vitamin B6 (dose range from 10 to 900 mg/day); 11 were supplemented with methionine (dose range from 100 to 1500 mg/day. Riboflavin (n=4; from 9 to 20 mg/day) and carnitine (n=4; from 1000 to 1500 mg/day) were rarely used. None of the patients treated with riboflavin carried mutations associated with in vitro FAD responsiveness.

Genetic and enzymatic data

Mutations, residual enzyme activity and age at first symptoms are listed in Table 2. The majority of mutations are private. Neither type nor location of mutation correlates with age at onset and pattern of clinical symptoms in this cohort. The common *MTHFR c.677* SNP has been studied in 32 patients. The c.677 SNP status did not contribute to classification of "low" or "higher residual" enzymatic activity: (low activity: 65 % CC, 14 % CT, 21 % TT; higher residual activity: 47 % CC, 21 % CT, 32 % TT). In the higher residual activity group, SNP status did not significantly contribute to activity level (% wt; CC: 8.9±10.9, CT: 5.3±3.7, TT: 5.3±3.7; one-way ANOVA, p=0.63). Thus, the c.677 SNP does not seem to have an effect on enzyme activity.

Clinical presentation and course in patients with very low (n=14) compared to higher residual enzyme activity (n=19)

Figure 2 depicts the different symptom patterns at presentation as solid columns and during the course (striped columns) in individuals with very low (black columns) compared to those with residual activity between 1.7 and 34.8 % (grey columns). Patients with higher enzyme activity showed predominantly psychiatric symptoms, confusion, mental retardation and neurological abnormalities such as myelopathy, ataxia and spasticity. In patients with low/absent enzyme activity, apnoea,

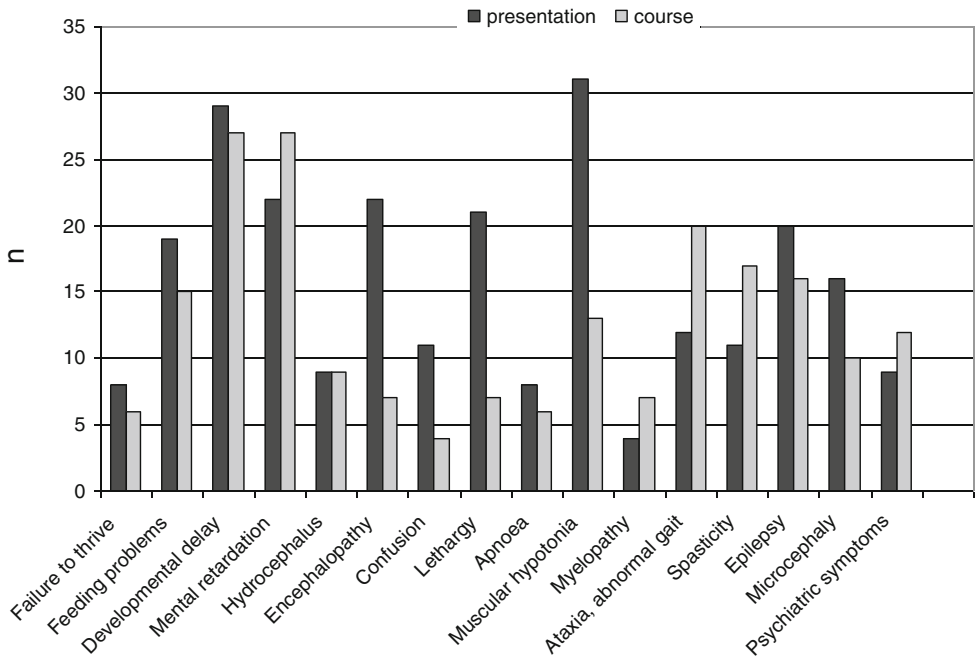

Fig. 1 Number of patients with specific clinical symptoms at presentation and during the course of the disease (n=33)

hydrocephalus or microcephaly and feeding problems occurred more frequently. It is of note, that the patient with residual enzyme activity as high as 34.8 % was symptomatic in terms of dementia and behavioural problems.

In addition, Fig. 2 allows the comparison between the frequencies of specific symptoms over time in both groups by comparing the respective solid and striped columns. Patients with very low enzyme activity more frequently developed failure to thrive, ataxia, spasticity, mental retardation, confusion and psychiatric disease during the course of their disease. However, all other symptoms remained stable or improved over time, in particular encephalopathy and muscular hypotonia. In patients with higher enzyme activity, frequencies of myelopathy, ataxia and spasticity slightly increased while all other symptoms remained stable or even improved over time.

Discussion

This cohort of 33 patients illustrates the spectrum of clinical manifestations of severe MTHFR deficiency and provides follow-up data over a long period of time (mean time of follow-up 9.2 years). However, it has to be emphasised that this study has clear limitations owing to its retrospective, proxy-reported design and the biased manner of recruitment of physicians from the senders of diagnostic material to a single diagnostic laboratory. Additionally, the cut-off for the separation of patient groups into "very low" and "higher" residual in vitro MTHFR deficiency groups is arbitrary in terms of possible clinical relevance but had been chosen due to the practically relevance that it relates to the minimum level of activity needed to perform kinetic measurements in cells.

Analysis of data from the complete cohort reveals that disease onset in MTHFR deficiency occurs predominantly early in life following an uneventful pregnancy, delivery and perinatal adaptation. Patients with very low enzyme activity in fibroblasts present at a younger age. Although low Met and high tHcy are the biochemical hallmarks of the disease, their concentrations are not correlated with age at onset, genotype, residual enzyme activity or severity/pattern of clinical symptoms, which is consistent with previous reports (Tonetti et al 2003; Thomas and Rosenblatt 2005; Forges et al 2010; Lawrance et al 2011).

The central muscular hypotonia and acute encephalopathy seen in our cohort are common features in early-onset inborn errors of metabolism. However, the severe feeding problems as observed in this cohort are consistently reported in MTHFR deficiency and other remethylation disorders (Fischer et al 2014) as well as in nutritional vitamin B12 deficiency (Roschitz et al 2005) and seem to be a more specific finding. Thus, when such problems are identified in undiagnosed patients (especially in the presence of microcephaly) they should focus the clinicians' attention towards a disease from this group and prompt the assessment of tHcy. Apnoea occurs particularly frequent in patients with MTHFR deficiency, but the underlying pathophysiological mechanisms are not yet understood.

The predominance of neurological symptoms, cognitive impairment, white matter disease and brain atrophy in the individuals reported here underscores the fact that MTHFR deficiency is a severe disease affecting primarily the central nervous system (CNS). Brain disease is mainly attributed to defective myelinisation (Surtees et al 1991; Kishi et al 1994a, b; Strauss et al 2007) due to the cerebral deficiency of S-adenosylmethionine. S-adenosylmethionine derives from Met which is low in MTHFR deficiency due to impaired function on the folate cycle which is involved in the remethylation of tHcy to Met. In addition, elevated S-adenosylhomocysteine directly inhibits methyltransferases and thus intensifies the reduction of overall methylation capacity (Smith et al 2012).

Course and outcome of severe MTHFR deficiency are dominated by neurocognitive impairment in almost all patients and neurological sequelae such as ataxia, spasticity and seizures in many patients. These findings are in line with other reports (Thomas and Rosenblatt 2005; Fattal-Valevski et al 2000; Forges et al 2010; Strauss et al 2007; Diekman et al 2014).

Surprisingly, although retinopathy has been shown in a mouse model (Lawrance et al 2011) and eye involvement is frequent in other methylation disorders such as the cblC, cblE and cblG defect (Fischer et al 2014; Huemer et al 2014a, b), eye disease has rarely been reported in severe MTHFR deficiency (Ronge and Kjellman 1996). This study indicates that patients with severe MTHFR deficiency should regularly be

Table 1 tHcy, homocystine and Met concentrations at diagnosis and under treatment

Parameter units and reference ranges	At diagnosis			Under treatment		
	Median (range)	Mean	n	Median (range)	Mean	n
Plasma tHcy (5–15 μmol/L)	170 (30–316)	163	28	71 (23–127)	73	29
Free homocystine (μmol/L; normally undetectable)	29 (9–70)	32	14	3.1 (0.6–14)	5.7	8
Urine homocystine (μmol/mmol creatinine; normally undetectable)	29 (4–627)	113	13	–	–	3
Plasma methionine (10–39 μmol/L)	9 (0–29)	9	30	27 (11–89)	35	23

Table 2 Age at presentation, brain imaging reports, MTHFR activity in fibroblast extracts[a] and mutations[b] detected in *MTHFR* in 33 patients

ID (Reference)	Age at presentation (months)	Brain imaging	Residual in vitro enzyme activity+FAD[a]	Mutation allele 1 predicted amino acid change	exon / intron	Mutation allele 2 predicted amino acid change	exon / intron
Patients with very low MTHFR activity of <1.5 % of the mean control value							
20[f] (Steinmann et al 1984)	0.25	Enlarged ventricles	<1.5 %	c.188G>C p.Trp59Ser	exon 1	c.188G>C p.Trp59Ser	exon 1
82	0.2	Atrophy, WMD[c]	<1.5 %	c.349G>A p.Ala113Thr	exon 2	c.792+1G>T splice site	Intron 4
39	0.75	Atrophy hydrocephalus	<1.5 %	c.391C>T p.His127Tyr	exon 2	c.655_657del p.Lys215del	exon 4
36	2	Atrophy, WMD	<1.5 %	c.452A>C p.Gln147Pro	exon 2	c.452A>C p.Gln147Pro	exon 2
41	4	nd[d]	<1.5 %	c.452A>C p.Gln147Pro	exon 2	c.452A>C p.Gln147Pro	exon 2
60[f] (Tsuji et al 2011)	1	Hydrocephalus	<1.5 %	c.458_459delinsTT p.Gly149Val	exon 2	c.458_459delinsTT p.Gly149Val	exon 2
42	0.1	Atrophy, WMD	<1.5 %	c.559C>T p.Arg183*	exon 3	c.559C>T p.Arg183*	exon 3
44	0.5	normal	<1.5 %	c.779 T>A p.Ile256Asn	exon 4	c.1025 T>C p.Met338Thr	exon 5
40	0.5	nd	<1.5 %	c.1027 T>G p.Trp339Gly	exon 5	c.1027 T>G p.Trp339Gly	exon 5
48	6	Atrophy	<1.5 %	c.1027 T>G p.Trp339Gly	exon 5	c.1027 T>G p.Trp339Gly	exon 5
18	1	Atrophy hydrocephalus	<1.5 %	c.1420G>T p.Glu470*	exon 8	c.1420G>T p.Glu470*	exon 8
25	nd	nd	<1.5 %	c.1420G>T p.Glu470*	exon 8	c.1420G>T p.Glu470*	exon 8
22	2.5	nd	<1.5 %	c.1542G>A (p.Lys510=)/splicing	exon 8	c.1542G>A (p.Lys510=)/splicing	exon 8
51	1	Atrophy, hydrocephalus	<1.5 %	c.1542G>A (p.Lys510=)/splicing	exon 8	c.1542G>A (p.Lys510=)/splicing	exon 8
Patients with MTHFR activity of>1.5 % of the mean control value							
Cell lines with in vitro FAD responsiveness[e]							
32	3.4	WMD, abnormal gyration	10.4 %	c.482G>A p.Arg157Gln	exon 2	c.482G>A p.Arg157Gln	exon 2
55 (Forges et al 2010; younger sibling)	0.3	WMD	1.7 %	c.535G>A p.Ala175Thr	exon 3	c.1178G>A (p.Trp389*) /splicing	exon 6
33 (Bathgate et al 2012)	216	normal	17 %	c.596C>T p.Ala195Val	exon 3	c.596C>T p.Ala195Val	exon 3
Cell lines with reduced affinity for nicotinamide adenine dinucleotide phosphate (NADPH)							
30	132	Atrophy	9.9 %	c.-40_-41delTC-	5′ UTR	c.1727C>T p.Pro572Leu	exon 10
29	2	Atrophy	8.1 %	c.276_314dup p.Leu89_Pro101dup	exon 2	c.1528 T>G p.Tyr506Asp	exon 8
52	0.1	normal	6.3 %	c.1126A>G p.Lys372Glu	exon 6	c.1542+2 T>C p.Tyr512Trpfs*3	exon 8
54[f]	0.75	nd	7.4 %	c.1141C>T p.Arg377Cys	exon 6	c.1359+1G>A splice site	intron 7
31	60	WMD	34.8 %	c.1142G>A p.Arg377His	exon 6	c.1142G>A p.Arg377His	exon 6
10	nd	WMD	3.8 %	c.1274G>C p.Trp421Ser	exon 7	c.1420G>T p.Glu470*	exon 8
37	2.5	Atrophy hydrocephalus	1.8 %	c.1644+2 T>G splice site	intron 9	c.1644+2 T>G splice site	intron 9
12	1.25	WMD atrophy	3.7 %	c.1764+1G>T splice site	intron 10	c.1764+1G>T splice site	intron 10
14	nd	WMD	3.0 %	c.1764+1G>T splice site	intron 10	c.1764+1G>T splice site	intron 10
13[f]	1.25	Atrophy	2.5 %	c.1765-18G>A p.Asp585Glyfs*14	intron 10	c.1765-18G>A p.Asp585Glyfs*14	intron 10
26	24	Atrophy	3.5 %	c.1765-18G>A p.Asp585Glyfs*14	intron 10	c.1765-18G>A p.Asp585Glyfs*14	intron 10
49 (Urreizti et al 2010; Case 4)	1	Atrophy hydrocephalus	2.0 %	c.1780delC p.Leu590Cysfs*72	exon 11	c.1780delC p.Leu590Cysfs*72	exon 11

Table 2 (continued)

ID (Reference)	Age at presentation (months)	Brain imaging	Residual in vitro enzyme activity+FAD[a]	Mutation allele 1 predicted amino acid change	exon / intron	Mutation allele 2 predicted amino acid change	exon / intron
38	1	Atrophy hydrocephalus	3.1 %	c.1805 T>C p.Leu598Pro	exon 11	c.1805 T>C p.Leu598Pro	exon 11
Cell lines with normal affinity for NADPH							
4 (Tallur et al 2005)	156	WMD	9.7 %	c.148C>T p.Arg46Trp	exon 1	c.167G>A p.Arg52Gln	exon 1
35	6	WMD	2.3 %	c.148C>T p.Arg46Trp	exon 1	c.1982G>C p.*657Serext*50	exon 11
72	4	WMD	2.2 %	c.1332G>C p.Ser440=/splicing	exon 7	c.1644+2 T>G splice site	intron 9

[a] Activities are from Burda et al 2015, and are the mean activities in fibroblasts extracts assayed by a physiological forward assay in the presence of 75 μM of the cofactor flavin adenine dinucleotide (FAD), and expressed as % of the mean control value

[b] Mutations are from Burda et al 2015. The numbering of the nucleotides changes, as well as exons and introns, follows the original nomenclature of Goyette et al 1998 with +13 as the number of the A of the ATG initiation codon, except for the c.-40_-41delTC change within 5′ UTR for which the A of the ATG initiation codon is +1

[c] *WMD* white matter disease

[d] *nd* no data

[e] MTHFR activity in fibroblasts of patients 32, 33 and 55 showed in vitro FAD responsiveness, i.e. activity measured in the presence of 75 μM FAD was significantly higher than without added FAD

[f] Deceased patients

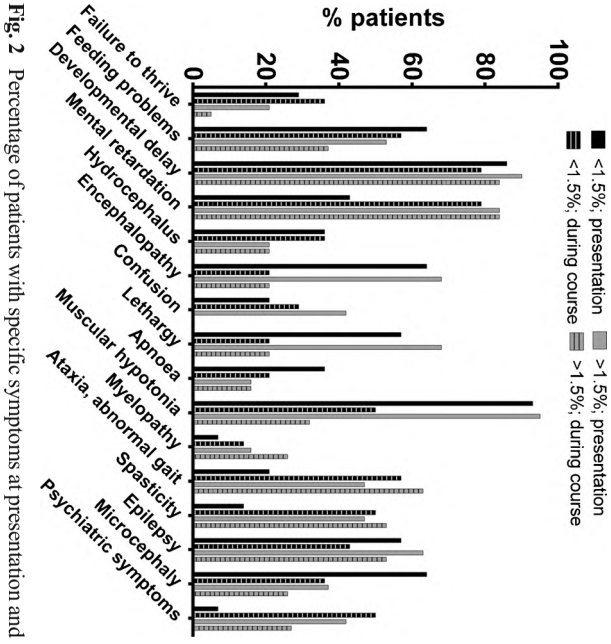

Fig. 2 Percentage of patients with specific symptoms at presentation and during the course by very low (<1.5 %; $n=14$) and residual (1.7–37 %) enzyme activity ($n=19$)

monitored for eye disease since nystagmus, visual impairment and optic atrophy were present in this cohort in 18 % of cases and symptoms were considered burdensome in 50 % of these patients. Folate deficiency, which is often associated with severe MTHFR deficiency, excessive oxidative stress induced by hyperhomocysteinemia or direct Hcy toxicity has been suggested to cause eye disease (Lawrance et al 2011). However, as in other remethylation disorders, the pathogenesis has not yet been completely elucidated and in many patients eye disease cannot substantially be alleviated by treatment (Weisfeld-Adams et al 2013). Macrocytosis and macrocytic anaemia, which are generally not considered part of the clinical spectrum of severe MTHFR deficiency (Watkins and Rosenblatt 2012), were reported in four patients and it remains unclear whether this finding is correlated with the underlying metabolic disorder or caused by other factors.

When stratifying the reported cohort according to very low and residual enzyme activity, two distinct patterns of disease presentation evolve. While patients with very low enzyme activity predominantly experience early-onset disease with severe neonatal encephalopathy often accompanied by apnoea, later-onset disease in patients with residual activity must be kept in mind as a differential diagnosis to mental retardation and psychiatric disease in children, adolescents and adults. This psychiatric type of the disease has occasionally been described before (Haworth et al 1993; Goyette et al 1995; Birnbaum et al 2008; Lossos et al 2014) and has been responsive to treatment in some cases (Birnbaum et al 2008; Michot et al 2008), which is consistent with our findings.

Although there was a clinical difference between patients with very low and residual MTHFR activity, we found no linear relationship between individual values of residual

activity, age at onset of first symptoms and disease course. Therefore it must be kept in mind that these in vitro studies are not to be used as prognostic markers in individual patients or families.

Correlations between type and location of mutation and enzyme level and kinetic characteristics had been observed in a study in fibroblasts of 72 patients, including the 33 patients presented here (Burda et al 2015). However, at least for the 33 patients reported here, no correlation between genotype and clinical phenotype could be delineated. The idea of phenotypic heterogeneity is also supported by the observation that siblings may present with a completely different clinically course (Haworth et al 1993). Furthermore, the common MTHFR c.677 SNP does not have an effect on enzyme activity.

In this cohort, treatment was found to normalise plasma Met, decrease tHcy concentrations and considerably improve or at least stabilise the clinical course. The response of brain disease detected by MRI (especially white matter damage) in response to treatment in the presented cohort is an important finding and in line with observations from single cases (Engelbrecht et al 1997; Al-Essa et al 1999). Similar resolution of white matter changes has been shown in treated patients with nutritional vitamin B12 deficiency (Ertan et al 2002) or the cblG defect (Outteryck et al 2012).

Generally, patients with higher residual activity seem to respond better to treatment. The overall disease load did not increase as significantly over time as in the very low activity group and mortality was less; this finding corresponds with the observations in the late-onset cblC defect (Huemer et al 2014b).

Treatment with betaine was applied to almost all, folate/folinate and hydroxycobalamin to the majority and methionine to many patients reported here. Since the study dates back to 1977 records of the exact dosages and time points of treatment initiation were often not available. However, since early betaine treatment has been recently shown to prevent neurocognitive decline in patients with severe MTHFR deficiency (Strauss et al 2007; Diekman et al 2014) we assume that most probably betaine and maybe methionine as an add-on treatment (Abeling et al 1999) have been the effective therapeutic agents in this cohort. In the present cohort, riboflavin has not systematically been applied to patients with in vitro FAD responsiveness and this may in the future be considered as an add-on trial.

Folate concentrations in serum and red blood cells as well as 5- methyltetrahydrofolate concentrations in cerebrospinal fluid (CSF) are generally low in severe MTHFR deficiency (Crushell et al 2012; Schiff and Blom 2012). However, treatment with CH3-tetrahydrofolate was neither able to normalise neurotransmitters (Schiff and Blom 2012) nor to alleviate the clinical course (Clayton et al 1986). Some authors argued in favour of a therapeutic effect for folinic acid on neurological symptoms (Crushell et al 2012) while others identified no clinical benefit (Holme et al 1989; Diekman et al 2014). In summary, the impact of folinic acid on clinical manifestations has not been convincing (Diekman et al 2014). Folic acid, which had been given to 19 patients in this cohort, should according to present knowledge not be applied in MTHFR deficiency since it may aggravate the deficiency of 5- methyltetrahydrofolate in the brain (Hyland et al 2010).

As in other inborn errors of remethylation, the older the patient at first symptoms, the longer the time until the correct diagnosis can be established. The pattern of an acutely ill neonate/infant with signs of neurological involvement seems likely to prompt timely metabolic workup, while an underlying inborn error of metabolism is not generally considered early in the diagnostic process in a child or adolescent with a more variable, non-specific psychiatric or neurological presentation (Huemer et al 2014a, b). Clinicians should be encouraged to actively seek severe MTHFR deficiency also in later presenting cases in order to shorten delay to diagnosis and allow timely initiation of treatment.

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Compliance with Ethics Guidelines

Conflict of interest None.

Human rights and informed concent All procedures followed were in accordance with the ethical standards of the responsible local committees on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000. Informed consent was obtained by the reporting physicians from their patients for being included in the study.

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