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ORIGINAL ARTICLE

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# Clinical and molecular characterization of adult patients with late-onset MTHFR deficiency

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**Abstract**

5,10-Methylenetetrahydrofolate reductase (MTHFR) deficiency usually presents as a severe neonatal disease. This study aimed to characterize natural history, biological and molecular data, and response to treatment of patients with

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late-onset MTHFR deficiency. The patients were identified through the European Network and Registry for Homocystinuria and Methylation Defects and the Adult group of the French Society for Inherited Metabolic Diseases; data were retrospectively colleted. To identify juvenile to adult-onset forms of the disease, we included patients with a diagnosis established after the age of 10 years. We included 14 patients (median age at diagnosis: 32 years; range: 11-54). At onset (median age: 20 years; range 9-38), they presented with walking difficulties (n = 8), cognitive decline (n = 3) and/or seizures (n = 3), sometimes associated with mild mental retardation (n = 6). During the disease course, symptoms were almost exclusively neurological with cognitive dysfunction (93%), gait disorders (86%), epilepsy (71%), psychiatric symptoms (57%), polyneuropathy (43%), and visual deficit (43%). Mean diagnostic delay was 14 years. Vascular events were observed in 28% and obesity in 36% of the patients. One patient remained asymptomatic at the age of 55 years. Upon treatment, median total homocysteine decreased (from 183 μmol/L, range 69-266, to 90 μmol/L, range 20-142) and symptoms improved (n = 9) or stabilized (n = 4). Missense pathogenic variants in the C-terminal regulatory domain of the protein were over-represented compared to early-onset cases. Residual MTHFR enzymatic activity in skin fibroblasts (n = 4) was rather high (17%-58%). This series of patients with late-onset MTHFR deficiency underlines the still unmet need of a prompt diagnosis of this treatable disease.

**KEYWORDS**
adult, inherited metabolic disease, late-onset, MTHFR deficiency, neurology

## 1 | INTRODUCTION

5,10-Methylenetetrahydrofolate reductase (MTHFR) deficiency (MIM #607093) is an autosomal-recessive disorder affecting the remethylation of homocysteine into methionine. MTHFR catalyzes an irreversible two-step reaction in which reducing equivalents are transferred first from nicotinamide adenine dinucleotide phosphate (NADPH) to the co-factor flavin adenine dinucleotide (FAD) and then passed on to 5,10-methylenetetrahydrofolate (methyleneTHF) forming 5-methyltetrahydrofolate (methylTHF).[1] It provides the only endogenous source of methylTHF, a critical methyl donor source in the remethylation of homocysteine into methionine through methionine synthase. Deficiency of the enzyme MTHFR leads to impaired provision of 5-methylTHF and decreased remethylation of homocysteine to methionine. Hyperhomocysteinemia is therefore characteristic of the disease.

MTHFR deficiency usually presents in the neonatal period with severe encephalopathy, microcephaly, seizures, feeding problems, and muscular hypotonia.[2,3] Apnoea is a frequent complication.[2,4-6] However, few late-onset (ie, after the age of 1 year)[2] and adult-onset forms of the disease have been reported, mainly as case reports.[7-22] Hence, the natural history of late-onset MTHFR deficiency is largely unknown (eg, pre-existing mild developmental delay, precipitating factors, and response to treatment). Disease onset and severity do not seem correlated with homocysteine levels at the time of diagnosis. A correlation between disease severity and residual enzyme activity has been suggested.[2,3] Although MTHFR deficiency is more easily diagnosed in its typical early-onset form (due to a greater probability of metabolic screening in critically ill children), patients with adolescent- or adult-onset are often misdiagnosed because of their less acute and nonspecific presentations

**Synopsis**

MTHFR deficiency can present in adults with gait difficulties, cognitive decline, epilepsy and/or psychiatric symptoms. Late-onset MTHFR deficiency is associated with pathogenic genetic variants and higher residual enzyme activity.

(eg, spastic paraplegia). It is therefore important to raise awareness and knowledge about milder forms of MTHFR deficiency in adult medicine. The aim of this work was to characterize natural history, biological and molecular data and response to treatment of adult patients with late-onset MTHFR deficiency.

## 2 | METHODS

Late-onset MTHFR deficiency is defined as a disease with onset after the age of 1 year. In patients with severe neonatal MTHFR deficiency (ie, onset before 1 year of age), the mean time from onset to diagnosis is 17 months.[2] In this series, we arbitrary decided to include patients with a diagnosis after the age of 10 years. Patients were identified through a research proposal communicated to the European Network and Registry for Homocystinuria and Methylation Defects (E-HOD) and the Adult group of the French Society for Inherited Metabolic Diseases (SFEIM-A). The study was approved by the E-HOD executive board. The patients included in the E-HOD registry agreed for collection and sharing of their data for scientific purposes. We retrospectively collected clinical, radiological, genetic, and biological data related to these patients. Data about MTHFR enzymatic activity in skin fibroblasts[23] were also collected, when available.

## 3 | RESULTS

We included 14 patients (from 12 families) with late-onset and genetically confirmed MTHFR deficiency for whom clinical and biological data were available (nine from France, two from the United Kingdom, one from the Netherlands, one from Germany, and one from the Czech Republic). Data on the epileptic presentation from patient #4 have been already published.[21] The diagnosis of MTHFR deficiency was based on the presence of pathogenic variants in the *MTHFR* gene in the context of compatible biological abnormalities (high level of total homocysteine, with low methionine) and clinical phenotype (with the exception of the asymptomatic individual). The median follow-up from disease onset to the last visit was 16 years (range 2-35 years). Demographic data are detailed in Table 1.

In most patients, the first clue to reach diagnosis was increased plasma homocysteine, measured because of the suspicion of a metabolic disease. Two patients (10# and 12#) received first a molecular diagnosis (through gene panel for hereditary spastic paraplegia and whole exome analysis, respectively) that was subsequently confirmed by

**TABLE 1** Demographic data of patients with late-onset MTHFR deficiency

| N | Sex/year of birth | Consanguinity | Family history | Study qualification | Job | Housing | FU (Years from onset to last visit) |
|---|---|---|---|---|---|---|---|
| 1 | F/1977 | Yes | Sister affected | Elementary school | Cleaning lady | Independent | 33 |
| 2 | F/1966 | No | Twin sister affected | NVQ level 1 | Cleaning lady | Dependent | 28 |
| 3 | M/1987 | Yes | No | NA | Sheltered work | Dependent | NA |
| 4 | M/1997 | Yes | Brother affected #14 | NVQ Level 3 | Student | Independent | 9 |
| 5 | M/1973 | No | No | Middle school | Lorry driver | Independent | 5 |
| 6 | M/1983 | Yes | Sister affected | Middle school | Sheltered work | Dependent | 14 |
| 7 | M/1994 | NA | NA | NA | None | Dependent | 17 |
| 8 | F/1970 | No | Her brother died before the age of 50 due to unknown leukoencephalopathy | NVQ Level 1 | None | Partially dependent | NA |
| 9 | F/1981 | NA | NA | NA | None | Dependent | 33 |
| 10 | F/1965 | Yes | Sister affected #11 | NA | None | Dependent | 34 |
| 11 | F/1964 | Yes | Sister affected #10 | NA | None | Dependent | 35 |
| 12 | F/1992 | No | No | High School | Clerk | Independent | 2 |
| 13 | M/1964 | No | No | High school | Technician | Independent | 3[a] |
| 14 | M/1999 | Yes | Brother affected #4 | NVQ Level 1,2 | Student | Partially dependent | 15 |

Abbreviations: F, female; FU,follow-up; M, male; NA, not available/assessed; NVQ level,National Vocational Qualification Level.

[a]Three years from diagnosis (and not from onset) as the individual is asymptomatic.

**TABLE 2** Clinical data of patients with late-onset MTHFR deficiency

| N | Onset (y when available)/ symptom at onset | Diagnosis (y)/ symptoms at diagnosis | A/SA/SP onset | Clinical symptoms throughout all the disease course | | | | | | | | | Cerebral MRI |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Paraparesis | Ataxia | Cognitive | Epilepsy | Psychiatric | PNP | Ophthalmological findings | Vascular events | Body Mass Index ($kg/m^2$) | |
| 1 | Mild mental retardation | 32/confusion, psychiatric, vascular, visual, gait | SA | Yes | Yes (sensory) | Yes | No | Yes | Yes | Optic atrophy, cataract, strabismus | Leg DVT | 37.7 | Cortical atrophy, WMH |
| 2 | 22/walking "pseudo MS" | 45/gait, cognitive, psychiatric, epilepsy | A | Yes | N | Yes | Yes | Yes | No | No | No | 33 | WMH |
| 3 | 12/cognitive, gait | 21/gait, cognitive | SP | Yes | No | Yes | Yes | No | No | No | No | 28.7 | NA |
| 4 | 11/cognitive, gait | 18/epilepsy | A | Yes | Yes (sensory) | Yes | Yes | No | Yes | No | No | NA | WMH |
| 5 | 38/cognitive, gait | 42/confusion, epilepsy | A | Yes | Yes | Yes | Yes | Yes | No | No | No | 36 | Cortical atrophy, WMH |
| 6 | 9/mental retardation, gait | 12/cognitive, psychiatric | SA | Yes | No | Yes | Yes | Yes | Yes | No | No | NA | WMH |
| 7 | Mental retardation, seizure | 11/psychiatric, cognitive | SP | NA | NA | Yes | Yes | Yes | No | No | No | 38 | Cerebellar atrophy, WMH |
| 8 | Mild mental retardation | 48/cognitive, gait | SP | Yes | No | Yes | Yes | No | Yes | Not specified reduced visual acuity | No | 24.9 | WMH |
| 9 | Mental retardation | 25/gait, mental retardation | SP | Yes | Yes | Yes | No | No | Yes | Lens luxation | No | 25 | NA |
| 10 | 20/gait, seizure | 53/increase of WMH | SP | Yes | No | Yes | Yes | Yes | No | Bilateral cataract | Stroke like (30 y) | 26 | WMH |
| 11 | 20/gait, seizure | 54/gait, diagnosis in a sib | SP | Yes | NA | Yes | Yes | Yes | No | Retinitis pigmentosa (partially blind) | Stroke (20 y) | 28 | WMH |
| 12 | 25/gait | 26/gait | SP | Yes | No | Yes | No | Yes | No | No | No | 25.5 | Cortical atrophy, WMH |
| 13 | Asymptomatic | 52/NA | NA | No | No | No | No | No | No | No | No | 28.9 | NA |
| 14 | Mental retardation | 16/epilepsy, diagnosis in a sib | A | No | Yes | Yes | Yes | No | Yes | Left optic neuropathy (subclinical) | Leg DVT | 36 | Cerebellar atrophy, WMH |

Abbreviations: A, acute; DVT, deep venous thrombosis; N, normal; NA, not available/assessed; PNP, polyneuropathy; SA, subacute; SP, slowly progressive; WMH, white matter hyperintensities.

**TABLE 3** Treatment and molecular and biological data of patients with late-onset MTHFR deficiency

| N | Initial treatment | Chronic treatment | Evolution | Total Hcy (Pre) µmol/L | Total Hcy (Post) µmol/L | Nucleotidic[a]/amino acid change | MTHFR activity on skin fibroblasts |
|---|---|---|---|---|---|---|---|
| 1 | Betaine 9 g/d; B12 1 mg/d os; folic a. 5 mg bid; B6 250 mg tid | Betaine 12 g/d; CyanoCbl 1 mg/wk os; folic a. 5 mg/d; B6 250 mg tid | After 6 wk better (behavior; gait; visual; cognition) | 250 | 92 | c.1130G > A/p.Arg377His<br>c.1130G > A/p.Arg377His | NA |
| 2 | Betaine 12 g/d; OHCbl 1 mg/d per os; folic a. 50 mg/d; B6 250 mg tid | Betaine 12 g/d; OHCbl 1 mg x 3/wk IM; folinic a. 25 mg bid; B6 250 mg tid | Better (behavior; gait) | 266 | 58 | c.197C > T/p.Pro66Leu<br>c.470G > A/p.Arg157Gln | 1.97 nmol/h/mg protein (patient); 6.10 nmol/h/mg protein (control); ratio 32% |
| 3 | NA | NA | Better (epilepsy; gait) | 246 | 110 | c.1130G > A/p.Arg377His<br>c.1130G > A/p.Arg377His | 4.61 nmol/h/mg protein (patient); 7.91 nmol/h/mg protein (control); ratio 58% |
| 4 | Betaine 6 g/d; OHCbl 1 mg/wk IM; folinic a. 5 mg bid; B6 250 mg bid | Betaine 6 g/d; OHCbl 1–2 mg/wk IM; folinic a. 5 mg bid; B6 250 mg bid | Better (epilepsy; gait) | 220 | 137 | c.1603C > T/p.Arg535Trp<br>c.1603C > T/p.Arg535Trp | NA |
| 5 | Betaine 6 g/d; OHCbl (low compliance); folinic a. 15 mg/d | Betaine 9 g/d; OHCbl 1 mg/wk IM; folinic a. 15 mg bid | Better (cognition; gait) | 126 | 60 | c.1899G > T/p.Trp633*<br>c.1162C > T/p.Arg388Cys | NA |
| 6 | Betaine 8 g/d; OHCbl 1 mg/wk IM; folinic a. 10 mg bid; methionine 800 mg/d | Betaine 8 g/d; OHCbl 1 mg/wk IM; folinic a. 10 mg bid; methionine 400 mg/d | Better (epilepsy; psychiatric and cognitive symptoms). Gait stable. | NA | 45 | NA | NA |
| 7 | OHCbl 1 mg/j IM; folic a. 15 mg/d; B2 10 mg/d | Betaine 6 g/d; OHCbl 1 mg/wk IM; folic a. 5 mg bid; B2 10 mg/d | Better (epilepsy; behavior). Stable mental retardation | 180 | 142 | c.469C > T/p.Arg156Trp<br>c.727 T > G/p.Cys243Gly | NA |
| 8 | Betaine 6 g/d; cyanoCbl 1 mg/twice a week per os; folinic acid 15 mg/d | Betaine 6 g/d, per os; cyanoCbl 1 mg/twice a week per os; folinic a. 15 mg/d | NA | 141 | 90 | c.359G > A/p.Cys120Tyr<br>c.1853 T > C/p.Leu618Pro | NA |
| 9 | Betaine 9 g/d; OHCbl 1 mg/mo; folic a. 5 mg/d; B6 250 mg; riboflavin 30 mg | Betaine 9 g/d; OHCbl 1 mg/mo; folic a. 5 mg/d; B6 250 mg; riboflavin 30 mg | Better (mood and behavior). Persistent spasticity. | 186 | 100 | c.1130G > A/p.Arg377His<br>c.1166 + 5G > C/--- | 1.5 nmol/h/mg protein (patient); 3.9-8.6 nmol/h/mg protein (control); ratio 17–38% |
| 10 | OHCbl 1 mg/mo; folic a. 5 mg/d | OHCbl 1 mg/mo; folic a. 5 mg/d; B6 50 mg bd (self-medicating) | Stable | 69 | 40 | c.459C > G/p.Ile153Met<br>c.1142G > A/p.Trp381* | NA |

(Continues)

**TABLE 3** (Continued)

| N | Initial treatment | Chronic treatment | Evolution | Total Hcy (Pre) µmol/L | Total Hcy (Post) µmol/L | Nucleotidic[a]/amino acid change | MTHFR activity on skin fibroblasts |
|---|---|---|---|---|---|---|---|
| 11 | NA | Betaine 6 g/d; OHCbl 1 mg/mo; folic a. 5 mg/d | Stable | 171 | 20 | c.459C > G/p.Ile153Met<br>c.1142G > A/p.Trp381* | NA |
| 12 | Betaine 6 g/d, OHCbl (1 mg/mo i.m.) | Betaine 6 g/d | Better (gait spasticity; WMH at MRI) | 216 | 110 | c.1006C > A/p.Leu336Met<br>c.1699C > T/p.Arg567* | NA |
| 13 | Folic a. 10 mg/wk | Folinic a. 15 mg/d | Stable. Asymptomatic. | 85 | 43 | c.1103C > A/p.Ala368Asp<br>c.1167-1G > A/--- | 4.38 nmol/h/mg protein (patient); 15 nmol/h/mg protein (control); ratio 29% |
| 14 | Betaine 9 g/d; OHCbl 1 mg IM/wk; folinic a. 5 mg bid; B6 250 mg | Betaine 9 g/d; OHCbl 1 mg × 2 IM/wk; folinic a. 5 mg bid; B6 250 mg | Stable | 166 | 89 | c.1603C > T/p.Arg535Trp<br>c.1603C > T/p.Arg535Trp | NA |

Abbreviations: bid, twice a day; folic a., folic acid; folinic a., folinic acid; Hcy, homocysteine; IM, intramuscular; MRI, magnetic resonance imaging; NA, not available/assessed; OHCbl, hydroxyCbl; tid, three times a day; WMH, white matter hyperintensities.

[a]According to HGVS; NM_005957.4.

the presence of increased plasma homocysteine. In patients 11# and 14#, plasma homocysteine was measured after the diagnosis of MTHFR deficiency in one sibling. In the asymptomatic individual (#13), the diagnosis was incidental during a regular work-up in the lipid clinic.

Median age at onset was 20 years (range 9-38 years) while median age at diagnosis was 32 years (range 11-54 years) (Table 2). Patient #13 was diagnosed at the age of 52 while still asymptomatic (incidental finding), and his case is briefly presented below. In our series, the earliest symptoms were: walking difficulties due to spasticity or ataxia (n = 8), cognitive decline (n = 3) and/or seizures (n = 3), sometimes associated with mild mental retardation (n = 6) (Table 2). These symptoms were mostly slowly progressive, not considered critical and occurred years before diagnosis. In most patients, MTHFR deficiency was finally suspected when the disease became increasingly severe and/or new symptoms occurred. At diagnosis, almost all patients presented with a combination of neurocognitive manifestations, and the main symptoms leading to diagnosis were: (a) cognitive decline and confusion (n = 7), with an acute (n = 1/7), subacute (n = 3/7), or slowly progressive (n = 3/7) presentation; (b) walking difficulties (n = 7), often slowly progressive (5/7 patients); (c) epilepsy (n = 4); (d) psychiatric symptoms (n = 4), occurring with an acute (n = 1/4), subacute (n = 2/4) or slowly progressive presentation (n = 1/4); (e) vascular event with deep venous thrombosis (n = 1); (f) increased white matter hyperintensities on brain magnetic resonance imaging (MRI) in the context of chronic spastic paraplegia (n = 1); and/or (g) reduced visual acuity (n = 1) (Table 2).

Globally, patients with adult late-onset MTHFR had almost exclusively neurological symptoms (Table 2). Over the course of their disease, most patients presented cognitive dysfunction (n = 13/14, 93%), gait disorders (n = 12/14, 86%)—mostly spastic paraplegia, sometimes sensory ataxia—, epilepsy (n = 10/14, 71%), and psychiatric symptoms (n = 8/14, 57%). A polyneuropathy documented by nerve conduction studies was present in 6 of 14 patients (43%). Visual impairment was reported in six patients (43%)—bilateral cataract (n = 2), lens luxation (n = 1), optic neuropathy (n = 1), retinitis pigmentosa (n = 1), and unspecified (n = 1). Vascular events were reported in four patients (28%)—leg deep venous thrombosis (n = 2), stroke-like or stroke events (n = 2). Interestingly, obesity (=BMI > 30 kg/m$^2$) with a BMI between 33 and 38 kg/m$^2$ was reported in 5 of 14 patients (36%) and overweight (BMI 25-30 kg/m$^2$) in another 6 of 14 patients (43%). At least one brain MRI was available from 11 of 14 patients and showed white matter abnormalities, with a prevalent posterior distribution, variably associated with cortical or cerebellar

atrophy (Table 2 and Figure S1). Spinal MRI was available for four patients and was normal (data not shown).

Details on initial treatment were available for 12 of 14 patients (Table 3). Betaine (6-12 g/day) was initially given to only 9 of 12 patients. Almost all patients (n = 11/12) received vitamin B12—mostly hydroxycobalamin (OHCbl), at very different dosages and route of administration—and/or folic/folinic acid (n = 11/12), variably associated with B6, B2, and methionine. Details on chronic treatment were available for 13 of 14 patients: betaine was given to 11 of 13 patients (6-12 g/day); folic/folinic acid (n = 12/13) and B12 (n = 12/13) were continued in almost all patients. One patient received B12 and folic acid without betaine and another patient folinic acid only. Seven patients required antiepileptic drugs (data not shown). At diagnosis, mean total homocysteine was 182 ± 63 μmol/L (range 69-266 μmol/L) while five patients had moderately low plasma folate levels and two patients decreased plasma B12 levels (data not shown). After treatment, mean total homocysteine decreased to 81 ± 37 μmol/L (range 20-142 μmol/L) and most patients (n = 9) experienced a clinical improvement with a notable regression of the symptoms that had developed during acute deterioration (confusion, psychiatric decompensation, walking deterioration, and seizures). Most chronic symptoms, such as mild developmental delay, persisted but stabilized (n = 4) (Table 3).

The presence of two pathogenic *MTHFR* variants was confirmed in all patients, although molecular data were incomplete for one patient. Most patients harbored private pathogenic variants (16 different variants in 12 families; homozygous variants in three families), but a few variants were recurrent (c.1130G > A) (Table 3). We found mostly missense variants, located in both the N-terminal catalytic (amino acids 1-356) and in the C-terminal regulatory domain (amino acid 363-656) of the protein. Nonsense or splicing mutations were less represented (n = 5), always associated with a missense variant, and located only in the C-terminal regulatory domain (Table S1). Enzymatic activity on skin fibroblasts was available for 4 of 14 patients (one analysis performed in the laboratory of the Brabois Hospital, in Nancy, France; three performed in the laboratory of University Children's Hospital in Zurich) and confirmed a mildly reduced MTHFR enzyme activity (from 17% to 58%, compared with controls) (Table 3).

### 3.1 | Case report of an asymptomatic individual incidentally diagnosed at the age of 52 years (Patient #13)

This is a 55-year-old male with incidental finding of hyperhomocysteinemia at the age of 52. His family history and childhood development were unremarkable. His neurological examination and cognitive functioning were both normal. At the age of 49 years, he underwent adrenalectomy for primary hyperaldosteronism (Conn syndrome). He was also followed for dyslipidemia (treated with statins) and asymptomatic hyperuricemia (treated with allopurinol). Hyperhomocysteinemia (plasma total homocysteine 85 μmol/L, reference range 3.5-15) was detected during a regular work-up in the lipid clinic. Until now, he has not developed any neurological symptom or thromboembolic event. Further laboratory findings at diagnosis were normal including plasma methionine, plasma cystathionine and undetectable methylmalonic acid in urine. Plasma B12 was in the normal range while serum folate was decreased (2.1 μg/L, reference range 3.9-26.8). He was supplemented with oral folic acid, which normalized serum folate level, and reduced but failed to normalize plasma homocysteine (plasma total homocysteine 43 μmolL/L at last follow-up). Molecular genetic investigation using a next generation sequencing panel of 56 genes associated with hyperhomocysteinemia revealed two variants with predicted pathogenicity—a splicing mutation c.1167-1G > A and a new missense variant c.1103C > A (p.Ala368Asp)—in the *MTHFR* gene. Segregation analysis in the family confirmed that the two alleles were in *trans*. Enzymatic analysis of MTHFR activity in skin fibroblasts was clearly decreased (4.38 nmol/mg prot/h; reference range 13.8-53.1).

## 4 | DISCUSSION

This is, to our knowledge, the largest series of adult MTHFR deficient patients with a late onset (median age at onset of 20 years). While initial symptoms were mainly represented by gait alterations and slight to moderate cognitive difficulties, they were often not perceived as being caused by an underlying condition or were undetected. Without treatment, patients deteriorated with cognitive decline, worsening of gait difficulties, seizures, and psychiatric symptoms, finally leading to the diagnosis of MTHFR deficiency. The mean delay between first symptoms and diagnosis was quite long—about 14 years. Therefore, it is critical for pediatricians to keep late-onset and slow progressive forms of the disease in mind, and for adult physicians to be aware of acute and chronic presentations of MTHFR deficiency. Moreover, we presented the case of an asymptomatic individual incidentally diagnosed as having MTHFR deficiency at the age of 52 years and remaining asymptomatic until now. Another individual still asymptomatic at the age of 37 years was previously reported.[9] Despite being less severe and less rapidly progressive than the classical

early-onset forms, late-onset MTHFR deficiency can also result, if untreated, into a neurodegenerative disorder and disabling handicap. In this series, only 5 of 14 patients were independent while 9 (64%) were totally or partially dependent.

As reported for patients with onset after 1 year of age,[24] our MTHFR deficient patients exhibited mainly neurological symptoms with cognitive and gait difficulties, associated with prevalent posterior white matter abnormalities on brain MRI. However, psychiatric symptoms (57% vs < 30%) and seizures (71% vs 35%) were much more prevalent in our series. Thromboembolic events were reported in 28% of our patients, in line with the 20% to 50% in other series.[8,19,20,24] Visual impairment can be observed in MTHFR deficiency, although less frequent and of various origins compared to other remethylation disorders.[25] Here, 43% of patients had ophthalmological manifestations, which suggests that they should be carefully monitored in MTHFR deficiency. Interestingly, 43% of patients were overweight, and obesity—that is, BMI between 33 and 38 $kg/m^2$—was observed in another 36% of this series. This is higher than in middle-aged European people where obesity is reported in about 16% of the population.[26] This finding has never been reported before and it is currently difficult to establish a direct link with MTHFR deficiency.

Even in this group of patients with late diagnosis and chronic symptoms, metabolic intervention was able to decrease total homocysteine levels, improve most of acute manifestations, and stabilize chronic symptoms. Unfortunately, in this retrospective series, it was not possible to describe the exact time course of homocysteine decrease and clinical improvement. According to the most recent international guidelines,[25] betaine is the only treatment with a proven efficacy[4] while folinic acid, vitamin B12, methionine, L-carnitine have yet to show efficacy (except for patients with deficiencies), and folic acid and protein restriction should be avoided because of the possible aggravation of cerebral methylTHF and methionine deficiency. In this series, most patients received betaine, except for two patients who had moderate hyperhomocysteinemia (<100 μmol/L) at diagnosis: one of them (#10) was clinically stable and total homocysteine decreased to 40 μmol/L after a treatment combining folic acid and vitamin B12; the other individual (#13) was asymptomatic and betaine therapy was refused by insurance company despite the intention to treat.

As already reported for severe MTHFR deficiency,[3,27] most patients harbored private pathogenic variants. However, the c.1130G > A variant was found in three different families and was already reported in patients with mild presentation or late-onset MTHFR disease.[3,17] Variants c.1162C > T, c.459C > G, c.470G > A, and c.1699C > T were previously reported in patients with late-onset deficiencies.[9,13,18,20,28] Interestingly, none of the variants most frequently recurring in patients with severe early-onset MTHFR deficiency p.Lys510 (c.1530G > A), p.Arg377Cys (c.1129C > T), p.Arg52Gln (c.155G > A), p.Arg157Gln (c.470G > A), p.Met338Thr (c.1013 T > C), and p.Trp339Gly (c.1015 T > G)[27] was found in this series. Therefore, our data suggest that some variants might be more frequent in late-onset forms, while others seem more frequent in early-onset cases. Missense pathogenic variants in the N-terminal catalytic domain and truncating variants in the C-terminal regulatory domain were previously associated with very severe phenotypes.[3] In our series (Table S1), we found that truncating mutations were located in the C-terminal regulatory domain (but never at the homozygous state) as well as many missense variants, unlike what was previously found in early-onset and severe patients.[3] This confirms the existence of a certain degree of genotype-phenotype correlation based on the nature of the variant and its location in functional domains of the enzyme.[3] Likewise, missense mutations in the C-terminal regulatory domain seems to be associated with higher residual enzyme activity and less severe clinical presentations.

Although not correlated with the severity of the disease, plasma homocysteine is an excellent diagnostic biomarker of MTHFR deficiency and it is increased even in subjects who are still asymptomatic. Likewise, plasma homocysteine should be measured in any child or adult with unexplained pyramidal signs, cerebellar syndrome, peripheral neuropathy, or seizures, especially if associated with intellectual disability and/or cognitive decline. Furthermore, the more appropriate parameter to predict disease severity and age at onset may be the degree of residual MTHFR enzymatic activity with marked reduced activity (<1.5%) in patients with severe presentations (early onset and/or death before 2 years), moderately reduced activity (1.7-11%) in patients with moderate to severe diseases, and mild or even no clearly reduced enzymatic activity (19%-42%) in patients with mild symptoms.[3] Intermediate to high residual activities were found in most[7,10,11,13,14,17] but not all[9,12,15,16,19] of the previously published patients with late-onset MTHFR deficiency. All the late-onset patients of this series for whom we measured MTHFR activity showed a high residual activity (from 17% to 58%, compared with controls), confirming the hypothesis of a correlation with disease severity. Other factors may also impact the disease course, such as FAD responsiveness and natural substrate affinity (NADPH, methyleneTHF, and FAD).[3]

This series has the limit of a retrospective study. However, it underlines the still unmet need of an early and prompt diagnosis of MTHFR deficiency in adults, a disease which is treatable with betaine. This study also contributes to an understanding of the different disease courses (ie, early- vs late-onset forms) of MTHFR deficiency with regard to molecular features and residual enzymatic activities.

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## CONFLICT OF INTEREST

Christian Lavigne, Karolina M. Stepien, Mirian C. H. Janssen, Francois Feillet, Viktor Kožich, Pavel Jesina, Rebecca Schule, Christoph Kessler, Isabelle Redonnet-Vernhet, Adeline Regnier, Patricie Burda, Matthias Baumgartner, Jean-Francois Benoist, and Fanny Mochel have nothing to declare. Cecilia Marelli has received travel and congress support from Biomarin. Martina Huemer has received support from Nutricia Metabolics for the development of patient education materials and a quality of life assessment tool for patients with intoxication type metabolic diseases. Martina Huemer has received consultancy or speaker's honoraria from Sanofi Genzyme, SOBI, Recordati Rare Diseases Foundation, Aeglea Biotherapeutics, and Orphan Europe. Conflicts of interest with the content of this work are not perceived.

## AUTHOR CONTRIBUTIONS

Cecilia Marelli participated in the study concept and design, acquisition of data, analysis and interpretation of the data, drafting and critical revision of the manuscript. Cecilia Marelli, Christian Lavigne, Karolina M. Stepien, Mirian C. H. Janssen, Francois Feillet, Viktor Kožich, Pavel Jesina, Rebecca Schule, Christoph Kessler, Isabelle Redonnet-Vernhet, Adeline Regnier, Patricie Burda, Matthias Baumgartner, and Jean-Francois Benoist participated in the acquisition of data and critical revision of the manuscript. Martina Huemer participated in the acquisition of data, critical revision of the manuscript and supervision of the study. Fanny Mochel participated in the study concept and design, acquisition of data, analysis and interpretation of the data, drafting and critical revision of the manuscript and supervision of the study.

## ETHICS STATEMENT

All authors were compliant and followed the ethical guidelines, according to the requirements of the JIMD. The patients included in the study agreed to collection and sharing of their data for scientific purposes.

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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