Reversal of Respiratory Failure in Both Neonatal and Late Onset Isolated Remethylation Disorders

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Abstract Respiratory failure is a well-documented potential presentation of inherited isolated remethylation disorders (IRDs). It appears to be a combination of both central and peripheral neuropathy and has previously often been considered to herald an irreversible neurological decline. We present three patients, one with methionine synthase (cblG) and two with methyltetrahydrofolate reductase deficiency (MTHFR). One patient with MTHFR presented in infancy, and other patients in later childhood. All three patients required intubation for respiratory failure but in all three, this was totally reversed by the initiation of medical therapy. This consisted of betaine and folinic acid supplementation in all three, methionine in two and cobalamin supplementation in two. The rate of respiratory improvement was variable, though two of the cases were successful extubated within a week of commencement of medical therapy. We document their subsequent clinical, biochemical and electrophysiological progress and review the potential pathological mechanisms underlying respiratory failure in these disorders.

Introduction

Isolated remethylation disorders (IRDs) are a rare, inherited group of enzymatic deficiencies, which result in impaired conversion of homocysteine to methionine without accumulation of methylmalonic acid. They include the rare Cobalamin D1 variant (cblD-HC) and deficient activity in the enzymes, Methyltetrahydrofolate reductase (MTHFR), methionine synthase (cblG) and methionine synthase reductase (cblE). While the exact pathological mechanisms are still to be fully elucidated, the neurological impairment seen in these disorders appears to be chiefly derived from the disturbance in the cellular methylation index (Weir and Scott 1995) and the toxic effects of homocysteine. Clinically IRDs can present with, or develop respiratory failure (de Ogier et al. 1998; Rosenblatt and Fenton 2001); with this often being heralded by an acute irreversible decline in overall clinical state (Narisawa et al. 1977; Nishimura et al. 1985; Al Tawari et al. 2002; Tsuji et al. 2011). Up to now, there have been extremely limited examples of successful therapeutic intervention (Surtees et al. 1991; Schiff et al. 2011). We document the resolution of the respiratory failure of three previously unreported patients with isolated remethylation defects (one methionine synthase deficiency and two MTHFR deficient patients). One patient with MTHFR deficiency presented neonatally, while the two other patients presented in later childhood. The respiratory failure in each case seems to have resulted from a combination of central and peripheral neurological impairment. We report on their presentation and response to therapy.

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Case Reports

Patient 1

Patient 1, diagnosed with methionine synthase deficiency both on complementation and functional fibroblast studies (MeCbl synthesis in fibroblast 16%, total reference range $58 \pm 18\%$), is the only child of an Asian father and Caucasian mother. After an uneventful antenatal course she was born at term weighing 2.44 kg. There were no initial perinatal concerns, but by 8 months of age she underwent genetic investigation for global developmental delay and generalised hypotonia. At 9½ years of age, when reviewed for two isolated seizures, she had ten distinct words, could run and scribble on paper but was still urinary incontinent. Reinvestigation at that time demonstrated a raised homocysteine and reduced methionine (see Table 1), a raised mean corpuscular volume (MCV) but normal plasma B12 and folate and urinary methylmalonic acid levels (MMA). A putative diagnosis of a remethylation disorder was made, however successful introduction of folinic acid and betaine were hampered by the patient's refusal to take oral medication.

While initiation of therapy was being attempted, her parents noted a decrease in her walking capacity and increasing lethargy. This worsened rapidly over the course of several weeks, culminating in her becoming unrousable. On admission to hospital, she had a partially compensated respiratory failure (pH 7.28, $PCO_2 = 13$ kPa, bicarbonate = 35 mmol/L), requiring ventilation. Extensive viral and bacterial investigations, including lumbar puncture, showed no evidence of infection. Her subsequent medication and biochemical responses to these are documented in Table 1. On admission to intensive care she had bilateral flaccid paralysis and absent deep tendon reflexes (DTRs) in her legs and limited antigravity movements with decreased reflexes in her arms. Diaphragmatic ultrasound indicated complete bilateral diaphragmatic palsy, while nerve conduction and electromyography (EMG) showed absent phrenic nerve responses and neurogenic changes bilaterally in both the intercostal muscles and the diaphragm. Further electrophysiological investigations showed undetectable sensory responses and only minimally recordable motor responses in her legs, while the upper limb and bulbar investigations also showed patchy neurogenic involvement. Her cranial MRI on admission showed no acute changes but a generalised lack of cerebral volume with some non-specific periventricular white matter changes. The magnetic resonance imaging (MRI) of her spine was normal.

Within 24 h of initiation of therapy to improve remethylation she was able to interact with her parents. She was extubated on day 5, though required 1 L of oxygen

and demonstrated marked tachypnoea and respiratory distress. Antigravity movements in her legs were noted on day 10. Polysomnography on day 14 showed a mean nocturnal transcutaneous CO_2 of 58 mmHg. After a month on therapy, she only required nocturnal oxygen supplementation and by 3 months post admission full polysomnographic examination was normal. Six months post admission she had regained all her previously acquired skills and had a completely normal physical examination.

Patient 2

Patient 2, a patient who has MTHFR confirmed on fibroblast studies (MTHFR activity 0.23 nmol/h/mg, control 25.3 ± 8.9 nmol/h/mg), was born at term, to non-consanguineous Asian parents, with no antenatal complications and weighed 2.98 kg. Again initial investigations for global developmental delay at a year were uninformative. At the age of 6½ years old, just prior to her clinical decompensation, she was fully fed orally, able to stand in a standing frame and to say her own name. Over the course of a 6-week period, she developed increasing sleepiness and poor feeding, culminating in respiratory failure, necessitating her transfer to the regional intensive care unit for ventilation. A cranial MRI on admission revealed a generalised global diffuse atrophy but no acute lesions. She was started on betaine, mefolinate and cyanocobalamin (see Table 1) on day 7 of her admission, when her raised homocysteine, low methionine and absence of urinary MMA or macrocytosis suggested MTHFR deficiency. After 48 h, she was alert and her previous apnoeic spells had improved markedly. Nerve conduction studies at onset of remethylation therapy demonstrated suppressed amplitude in both posterior tibial, median and ulnar nerves and her EMG findings were also consistent with decreased innervation. This corresponded to the clinical findings of profound hypotonia, areflexia and an MRC power assessment score of grade 1 in all four limbs. Her ventilatory support was initially weaned rapidly and extubation was first attempted at 48 h after betaine was introduced. However it was prevented by recurrent left lower lobe collapse, which occurred at low pressures. Thoracic CT and bronchoscopy excluded extrinsic compression and intrinsic obstruction, while diaphragmatic ultrasound indicated unilateral diaphragmatic palsy.

Twenty-one days post initiation of therapy the power in the patient's upper limbs had improved to an MRC score of 4 but the left diaphragmatic palsy continued. A tracheostomy allowed gradual weaning, with the patient coming off all ventilatory support at 127 days post initiation of therapy. Two years post her acute decompensation, she has normal respiratory function, reflexes and upper limb strength, though her lower limbs still show some residual weakness.

Table 1 Therapy and the biochemical responses

Time	Plasma THCy ($\mu\text{mol/L}$) [<15]	Plasma Met ($\mu\text{mol/L}$) [10–60]	CSF Met ($\mu\text{mol/L}$) [1–4]	CSF MTHF (nmol/L) [52–178]	Betaine (g/day)	B12 (mg/ day IM)	Calcium folinate (mg/day)	B6 (mg/day)	Met supplement (g/day)
<i>Patient 1: Methionine synthase</i>									
Pre Rx	140	14							
PICU day 0	78	14	4		8	1	15	200	
PICU Day 3	88	285			8	1	15	200	2
PICU Day 4	80	345			8	1	15		1
+8 months	61	54			8	1	15		1
	Plasma THCy ($\mu\text{mol/L}$) [5–15]					B12 (mg once per week)	Mefolate (mg/day)		
<i>Patient 2: MTHFR</i>									
Pre	269	7		0					
1 month	88	10			6	10	15		
2 month	80	6			6	10	15		
+1 year	123	18		18	6	10	15		
+2 years	113	24			6	10	15		
+3 years	111	21			6	10	15		
	Plasma THCy ($\mu\text{mol/L}$) [<15]					B12 (mg/ day)	Calcium folinate (mg/day)	Riboflavin (mg/day)	
<i>Patient 3: MTHFR</i>									
Pre Rx	166	16	1						
2 weeks	212	6						50	
3 weeks	115	24			4	1 IM	15		200
8 months	112	34			4		15		200
+2 years	123	19	1	10	4		15		200

The normal ranges for each of the metabolite are indicated in the square brackets. NB all medication given orally unless indicated

Patient 3

Patient 3 is homozygous for a novel splice site mutation c.1530G>A in the 3' splice site of exon 9 of MTHFR. This is predicted to be pathogenic by splice site prediction software MaxEntscan, NNSplice and Genesplicer. She is the second child of consanguineous Asian parents, whose only previous child had died of respiratory failure at 2 months of age, shortly after placement of a VP shunt for a Dandy–Walker malformation. After delivery at term, weighting 2.97 kg, she was initially well but developed increasing feeding difficulties from 2 weeks. At referral, for investigation of recurrent cyanotic spells at 4½ months of age, she had marked failure to thrive, global developmental delay and acquired macrocephaly. On examination she had

gross hypotonia with head lag, and minimal lower limb movement (MRC power score 3/5) but her DTR could be elicited. Given her hypotonia and respiratory weakness, she was initially started on riboflavin for possible Brown–Vialletto–Van Laere Syndrome. Subsequent biochemical investigations indicated an isolated remethylation disorder and her subsequent medication and biochemistry are noted (see Table 1).

Subsequent to a cranial MRI, which suggested obstructive hydrocephalus, she failed 2 extubations due to poor respiratory drive and a chest X-ray indicated a raised right hemidiaphragm, consistent with right phrenic nerve palsy. Nerve conduction studies showed prominent denervation with re-innervation in both upper and lower limbs and some bulbar impairment. She remained intubated for 7 days post

initiation of therapy and required CPAP for a further 1½ weeks. In total she required 3½ weeks of respiratory support. Her cyanotic episodes proved to be seizure related, this initially settled without specific seizure medication. A sleep study at 1 year of age was normal and gastroscopy was successfully inserted without complications. At 2 years of age she is starting to eat more and stand unsupported, however she now requires sodium valproate for seizure control.

Discussion

All three patients described here had remethylation defects and had varying degrees of respiratory compromise on presentation. Respiratory failure is a well-documented complication of isolated remethylation disorders (Roseblatt and Fenton 2001), being particularly prominent in children presenting between 3 months to 10 years though it can occur at any age (de Ogier et al. 1998). The pathophysiological mechanisms underlying this respiratory failure are poorly defined but on the evidence of the patients described here, appear to be multifactorial in nature, encompassing both central and peripheral neurological impairment with the combination of both ultimately leading to inadequate ventilation.

Peripheral neuropathy is a well-described late onset complication in IRDs, (Pasquier et al. 1994; Haworth et al. 1993; Tonetti et al. 2003; Chaabene-Masmoudi et al. 2009) but is also increasingly recognised in the infantile onset age group (Tsuji et al. 2011; Al Tawari et al. 2002). In this case series there was direct electrophysiological evidence of phrenic nerve dysfunction in patient 1, while patients 2 and 3 had radiological evidence strongly suggestive of diaphragmatic, i.e. phrenic nerve dysfunction. Also all three patients had other electrophysiological evidence of generalised peripheral nerve involvement. As was seen in patients 1 and 2 here, worsening lower limb neuropathy can herald potential respiratory decompensation (Nishimura et al. 1985; Kishi et al. 1994). The response of this peripheral neuropathy is often at best limited (Chaabene-Masmoudi et al. 2009; Schiff et al. 2011); however, in this case series complete electrophysiological resolution was documented in the infantile onset MTHFR case accompanied by normalisation of clinical symptomatology, while apparent clinical resolution was seen in patients 1 and 2.

Given the limited numbers and the retrospective nature of these cases, it is difficult to be certain as to why there seems to be such a greater improvement in peripheral nervous function between these cases and those previously described in literature. It is possible that the early introduction of

methionine may have played a role, with two of the patients described here, having methionine started as part of the initial attempt to normalise their biochemical milieu. Patient 2 who was not supplemented took the longest to resolve and indeed continued initially to have low levels of plasma methionine despite betaine supplementation, though low methionine has been documented in other patients even with methionine supplementation (Clayton et al. 1986; Chaabene-Masmoudi et al. 2009). It has previously been seen that methionine supplementation has resulted in a rapid improvement in a neonate with MFHTR already on betaine and folate (Abeling et al. 1999); with the suggestion that the betaine:homocysteine methyltransferase was inadequate to fully maintain remethylation. It was thought to also be beneficial in three patients in a recent case series by Schiff et al. (2011), however it is still not standard therapy for MTHFR and has not been frequently used historically. It is also possible that betaine therapy has in these patients been started early enough (Diekmann et al. 2014) to prevent the decrease in neuronal numbers that has been previously noted (Nishimura et al. 1985) and attributed to the reduction of myelin (Surtees et al. 1991). Certainly adequate concentrations of methionine are required to produce *S*-adenosylmethionine (AdoMet) (Surtees et al. 1991), the major methyl donors (Katz et al. 2003) with low levels of AdoMet being seen in a range of remethylation disorders (Surtees 1998). In turn methylation is crucial in myelin formation as it determines both the tertiary structure of myelin basic protein (Amur et al. 1986) and is vital for the production of phosphatidylcholine, a key constituent of the myelin sheath (Weir and Scott 1995).

It has also been suggested that that *S*-adenosylhomocysteine (AdoHcy), a potent inhibitor of methyltransferases (Schatz et al. 1981), shown to accumulate with high levels of homocysteine (Dayal et al. 2001; de la Haba and Cantoni 1959) may prevent methylation and inhibit myelination (Weir and Scott 1995). However these cases seem to suggest that this may not be as important a factor, given patient 3's improvement despite her persistent homocysteine levels >110 µmol/L.

The major presynaptic input for the phrenic nerve is derived from the dorsal and ventrolateral respiratory centres in the medulla. Thus central nervous system depression leading to presynaptic inhibition may also cause phrenic nerve inhibition. Central causes of respiratory failure seem to have been a major cause of in the early onset case of MTHFR reported here, with recurrent apnoeas occurring. This lack of central drive may have resulted from pressure effects since patient 3 did show ventriculomegaly suggestive of hydrocephalus. This has been described in neonatal MTHFR patients who presented with apnoea and required shunting (Tsuji et al. 2011; Baethmann et al. 2000). However the very

rapid improvement in this patient is hard to explain purely in terms of pressure effects. Other potential mechanisms for the improved respiratory drive would include the improvement of the low levels of brain choline seen in patients with remethylation disorders (Debray et al. 2008) or improved cerebral glucose uptake related to increase AdoMet levels (Al-Essa et al. 1999).

While it is likely that improved muscle function resulted from improved neurological function, all the patients had a period of inadequate nutrition prior to their admission to PICU. However the timing of the improvements in respiratory function all coincided with the introduction of medication targeted at improving remethylation, not with the institution of general supportive measures. It also is to be noted, that although the clinical manifestations of remethylation disorders are very similar (de Ogier et al. 1998), the levels of tetrahydrofolate/available cellular folate pool is reduced to a greater degree in MTHFR than CblG which may result in differing pathological cascades in the different remethylation disorders.

Summary

Respiratory failure in isolated remethylation disorders is likely to be multifactorial in nature with these cases suggesting that the prompt institution of appropriate therapy can result in its complete resolution. While the timeframe to complete resolution varied some definitive improvements were seen within the first couple of weeks of therapy in all patients.

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Synopsis

Acute respiratory failure seen in remethylation disorders is multifactorial but with aggressive medical management can be reversed.

Compliance with Ethics Guidelines

Alexander Broomfield, Lara Abulhoul, Matthew Pitt, Elisabeth Jameson and Maureen Cleary declare that they have no conflict of interest.

This article does not contain any studies with human or animal subjects performed by any of the authors.

Contributions of Each Author

Dr Broomfield conception and design of study, draft article. Dr Abulhoul revision of article and collection of data. Dr Jameson design of article and revision. Dr Pitt analysis and interpretation of data. Dr Cleary revision of article and provision of data.

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