

Table S1. Time-course of biomarkers and therapeutic regiment of four newborns with a positive newborn screen for MTHFR deficiency.

Patient 1														
	DBS		URINE		SERUM									
	I	II	4/20/18	11/7/18	3/7/18	11/7/18	2/5/19	5/30/19	10/29/19	3/3/20	10/6/20	4/19/21	4/19/22	9/27/22
Met: <i>cut-off DBS: 6-20 μmol/L</i> <i>rv serum: 10-60 μmol/L (<1 month)</i> <i>9-42 μmol/L (>1 month)</i>	4	6				10								26
C3: <i>cut-off DBS: 3.18 μmol/L</i> <i>rv serum: 0.04-3.18 μmol/L</i>	1.68					0.3								
Hcy: <i>cut-off DBS: 4 μmol/L</i> <i>rv serum: 5-15 μmol/L</i>	53.7	42.3			106.7	51.7	47.1	56.9	44.8	50.6	45.5	52.9	51.5	52.4
MMA: <i>rv urinary: nd-5 μmol/L</i>			4	2										
Maternal Vit B12: <i>rv serum: 197-866 pg/mL</i>														
Vit B12: <i>rv serum: 197-866 pg/mL</i>								502	586		390		526	540
Therapy*: <i>M: mg/die</i> <i>B12: mg/die (i.m.)</i> <i>B: mg/kg/die</i> <i>*M: Mefolate, B: Betaine</i>						M: 45 B12: 1 B: 250	M: 45 - B: 250	M: 45 - B: 250	M: 45 - B: 250	M: 45 - B: 250	M: 45 - B: 250	M: 45 - B: 250	M: 60 - B: 250	M: 60 - B: 250

DBS: Dry Blood Spot; Met: Methionine; C3: C3-Carnitine; Hcy: Homocysteine; MMA: Methylmalonic Acid.

Patient 2

	DBS		URINE		SERUM	
	I	II	3/9/20	5/28/20	3/9/20	5/19/20
Met: <i>cut-off DBS: 6-20 μmol/L</i> <i>rv serum: 10-60 μmol/L (<1 month)</i> <i>9-42 μmol/L (>1 month)</i>	5.5	9			41	31
C3: <i>cut-off DBS: 3.18 μmol/L</i> <i>rv serum: 0.04-3.18 μmol/L</i>	3.03				1.29	
Hcy: <i>cut-off DBS: 4 μmol/L</i> <i>rv serum: 5-15 μmol/L</i>	4.4	5.11			14.7	17.3
MMA: <i>rv urinary: nd-5 μmol/L</i>			38	37		
Maternal Vit B12: <i>rv serum: 197-866 pg/mL</i>					367	
Vit B12: <i>rv serum: 197-866 pg/mL</i>						
Therapy*: <i>FA: μg/die</i> <i>*FA: Folic Acid</i>					FA: 400	

DBS: Dry Blood Spot; Met: Methionine; C3: C3-Carnitine; Hcy: Homocysteine; MMA: Methylmalonic Acid.

Patient 3

	DBS		URINE		SERUM									
	I	II	12/2/20	3/25/21	11/4/20	12/2/20	1/22/21	3/25/21	6/15/21	9/21/21	12/10/21	3/23/22	7/22/22	11/4/22
Met: <i>cut-off DBS: 6-20 μmol/L</i> <i>rv serum: 10-60 μmol/L (<1 month)</i> <i>9-42 μmol/L (>1 month)</i>	5.07	8.6				29								
C3: <i>cut-off DBS: 3.18 μmol/L</i> <i>rv serum: 0.04-3.18 μmol/L</i>	0.89					0.7								
Hcy: <i>cut-off DBS: 4 μmol/L</i> <i>rv serum: 5-15 μmol/L</i>	11	16.3			53.1	41.9	33.6	28.9	30.3	26.5	28.2	25.0	27.9	28.8
MMA: <i>rv urinary: nd-5 μmol/L</i>			5	5										
Maternal Vit B12: <i>rv serum: 197-866 pg/mL</i>														
Vit B12: <i>rv serum: 197-866 pg/mL</i>						347	371	554	356	271	224	>2000	>2000	>2000
Therapy*: <i>M: mg/die</i> <i>B12: mg/die (o.s.)</i> <i>B: mg/kg/die</i> <i>*M: Mefolate, B: Betaine</i>						M: 45 - B:250				M: 45 B12: 0.33 B: 250				M: 45 B12: 0.33 B: 250

DBS: Dry Blood Spot; Met: Methionine; C3: C3-Carnitine; Hcy: Homocysteine; MMA: Methylmalonic Acid.

Patient 4

	DBS		URINE		SERUM	
	I	II	8/4/22	9/8/22	8/4/22	9/8/22
Met: cut-off DBS: 6-20 $\mu\text{mol/L}$ rv serum: 10-60 $\mu\text{mol/L}$ (<1 month) 9-42 $\mu\text{mol/L}$ (>1 month)	5.6	8			23	
C3: cut-off DBS: 3.18 $\mu\text{mol/L}$ rv serum: 0.04-3.18 $\mu\text{mol/L}$	3.02				1.5	0.3
Hcy: cut-off DBS: 4 $\mu\text{mol/L}$ rv serum: 5-15 $\mu\text{mol/L}$	9.2	7			54.5	3.7
MMA: rv urinary: nd-5 $\mu\text{mol/L}$			403	9		
Maternal Vit B12: rv serum: 197-866 pg/mL					143	
Vit B12: rv serum: 197-866 pg/mL					<83	>2000
Therapy*: B12: mg/die (o.s. 6 days/week and i.m. once/week) C: mg/kg/die *C: Carnitine					B12: 0.33 C: 100	

DBS: Dry Blood Spot; Met: Methionine; C3: C3-Carnitine; Hcy: Homocysteine; MMA: Methylmalonic Acid.