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Succinyl CoA

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Newborn screening for homocystinurias and methylation disorders: systematic review and proposed guidelines

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Abstract Newborn screening (NBS) is justified if early intervention is effective in a disorder generally not detected early in life on a clinical basis, and if sensitive and specific biochemical markers exist. Experience with NBS for homocystinurias and methylation disorders is limited. However, there is robust evidence for the success of early treatment with diet, betaine and/or pyridoxine for CBS deficiency and good evidence for the success of early betaine treatment in severe MTHFR deficiency. These conditions can be screened in dried blood spots by determining methionine (Met), methionine-to-phenylalanine (Met/Phe) ratio, and total homocysteine (tHcy) as a second tier marker. Therefore, we recommend NBS for cystathionine beta-synthase and severe MTHFR deficiency. Weaker evidence is available for the disorders of intracellular cobalamin

metabolism. Early treatment is clearly of advantage for patients with the late-onset cblC defect. In the early-onset type, survival and non-neurological symptoms improve but the effect on neurocognitive development is uncertain. The cblC defect can be screened by measuring propionylcarnitine, propionylcarnitine-to-acetylcarnitine ratio combined with the second tier markers methylmalonic acid and tHcy. For the cblE and cblG defects, evidence for the benefit of early treatment is weaker; and data on performance of Met, Met/Phe and tHcy even more limited. Individuals homozygous or compound heterozygous for *MAT1A* mutations may benefit from detection by NBS using Met, which on the other hand also detects asymptomatic heterozygotes. Clinical and laboratory data is insufficient to develop any recommendation on NBS

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for the cblD, cblF, cblJ defects, glycineN-methyltransferase-, S-adenosylhomocysteinehydrolase- and adenosine kinase deficiency.

Abbreviations

ADK	adenosine kinase
C2	carnitine, acetylcarnitine
C3	carnitine propionylcarnitine
CBS	cystathionine beta-synthase
DBS	dried blood spots
GNMT	glycine-N-methyltransferase
Hcy	homocysteine
MAT	methionine adenosyltransferase
MMA	methylmalonic acid
Met	methionine
MTHFR	methylenetetrahydrofolate reductase
NBS	newborn screening
OH-Cbl	hydroxycobalamin
R4S	Region 4 Stork
SAHH	S-adenosylhomocysteine hydrolase
tHcy	total homocysteine

Introduction

One of the major problems in the evaluation of data on clinical course and treatment efficacy in rare diseases is the absence of standardized databases derived from large numbers of patients assembled in an unbiased manner. Such data on natural course and benefits of early intervention are essential to support decisions on what to include in newborn screening (NBS) panels. To meet these needs for a specific group of inborn errors of metabolism, E-HOD, the European network and registry for homocystinurias and methylation defects (disturbed S-adenosylmethionine and S-adenosylhomocysteine ratio) was started in February 2013 (Table 1, pathways depicted in Fig. 1). Aims of the project are, among others, the establishment of a disease registry for the collection of clinical, biochemical and genetic data, and the development of guidelines.

The aims above are pursued in this report with respect to guidelines for NBS for the homocystinurias and methylation disorders developed on the basis of a systematic review of the literature, current NBS practice and expert discussions.

Methods

A systematic search of the Pubmed and Cochrane databases was performed using the search term “newbornscreening” in combination with every disease addressed by this report. To

evaluate treatment efficacy, publications on clinical course, outcome and treatment were searched for.

After exclusion of publications containing no relevant information, study design and level of evidence was evaluated for 103 publications according to the “Grading of Recommendations Assessment, Development and Evaluation (GRADE) working group (<http://www.gradeworkinggroup.org/>)” approach. Furthermore, data on metabolite concentrations in dried blood spots (DBS) at the time of screening obtained from 486 patients with subsequently confirmed homocystinurias and methylation disorders as well as cumulative data on distribution of analytes in healthy newborns and clinically validated target ranges for cut-off values were retrieved from the multi-center study “Region 4 Stork” (R4S) website (McHugh et al 2011). In addition, EHOD partners were asked to contribute their non-published experience with NBS for the disorders of interest. The guidelines were established based on the literature evaluation and expert discussions following the GRADE approach. Expert consensus was targeted for each disease on the following key questions:

- Is the natural course of the disease severe?
- Is treatment generally beneficial?
- Is early intervention more effective?
- Are robust, valid and reliable methods, screening approaches and strategies available?

Results

Classical homocystinuria or cystathionine- β -synthase deficiency

Clinical findings

In classical homocystinuria or cystathionine- β -synthase [CBS] deficiency, the condensation of serine and homocysteine (Hcy) to form cystathionine is disturbed as well as other processes such as the production of hydrogen sulphide from other substrates. Pyridoxal 5'-phosphate formed from dietary vitamin B₆ is the essential cofactor of the enzyme. The true incidence of CBS deficiency is unknown; it has been estimated at 1:344.000 worldwide (Mudd et al 1985) but due to different population frequencies and ascertainment methods, reports on CBS incidence vary largely. Molecular epidemiological studies predict frequencies between 1:6.400 and 1:20.500 in European populations (Gaustadnes et al 1999; Refsum et al 2004; Janosík et al 2009).

Biochemical hallmarks of CBS deficiency are highly elevated concentrations of plasma total homocysteine (tHcy) combined with low plasma cystine or total cysteine, high normal to elevated plasma methionine (Met), low normal to

Table 1 Metabolic disorders addressed in this report, number of mutations, R4S data and population frequency/estimated number of patients

Disorder, gene and chromosomal location	Mutations	Number of patients with laboratory data in R4S	Estimated number of patients/population frequency	ORPHA codes	MIM codes
Cystathionine-β-synthase deficiency (classical homocystinuria); <i>CBS</i> ; 21q22.3	187 ^a	124	>1000	394	613381
Severe <i>MTHFR</i> deficiency; 1p36.22	101 ^b	17	1:344,000 ^c	395	607093
Cobalamin E (CblE) defect <i>MTRR</i> ; 5p15.31	26 ^a		>85	2169	602568
Cobalamin G (CblG) defect <i>MTR</i> ; 1q43	22 ^a		~50	2170	156570
Cobalamin C (CblC) defect <i>MMACHC</i> ; 1p34.1	77 ^a	198	~50	79282	609831
Cobalamin D (CblD) defect; <i>MMADHC</i> ; 2q23.2	13 ^a		>300	79283	611935
			<20	308380	
			<20	308442	
Cobalamin F (CblF) defect; <i>LMBRD1</i> ; 6q13	9 ^a	4	<20	79284	612625
Cobalamin J (CblJ) defect; <i>ABCD4</i> ; 14q24.3	5 ^a	0	<10	369955	603214
Methionine adenosyltransferase (<i>MAT I/III</i>) deficiency; <i>MAT1A</i> ; 10q23.1	48 ^a	143	1:28,000 ^d	168598	610550
Glycine-N-methyltransferase (<i>GNMT</i>) deficiency; <i>GNMT</i> ; 6p21.1	3 ^a	0	3	289891	606664
S-adenosylhomocysteine hydrolase (<i>SAHH</i>) deficiency; <i>AHCY</i> ; 20q11.22	10 ^a	0	8	88618	613752
Adenosine kinase (<i>ADK</i>) deficiency; <i>ADK</i> ; 10q22.2	5 ^a	0	6	289290	102750

All disorders are inherited as autosomal recessive traits with the exception of *MAT I/III* deficiency, which may also be inherited as an autosomal dominant trait

^a Human Gene Mutation Database (www.hgmd.org)

^b Burda P, personal communication

^c Skovby et al. (2010)

^d Martins et al. (2012); Couce et al. (2013)

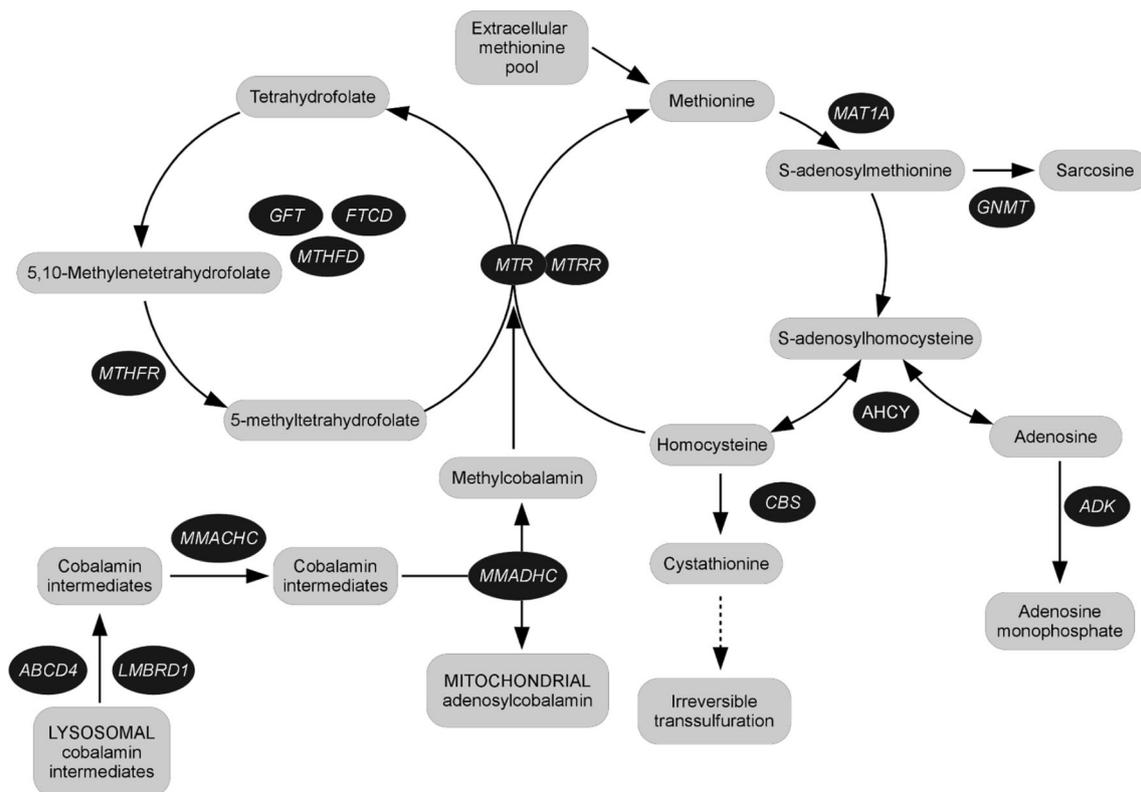


Fig. 1 Homocystinurias and methylation disorders: metabolic pathways

decreased cystathionine, and a grossly abnormal cystathionine/Met ratio (Stabler et al 2013; Bártl et al 2014). Generally, patients present with ectopia lentis and myopia, mental retardation, marfanoid habitus and osteoporosis, thromboembolic events, developmental and behavioral problems (Mudd et al 1985). If diagnosis is delayed, significant long-term morbidity must be expected (Naughten et al 1998; Yap and Naughten 1998). Milder forms and especially the pyridoxine-responsive variants of the disease due to specific mutations such as c.833 T>C (p.I278T) may manifest later in adulthood e.g., with thromboembolic events or may even remain asymptomatic until adulthood (Skovby et al 2010; Magner et al 2011).

In the pyridoxine-responsive patients, variable doses of pyridoxine elicit near-normalization of biochemical parameters (Mudd et al 1985). Patients with the more severe, pyridoxine non-responsive form of the disease are generally treated with a Met restricted diet mostly combined with supplementing amino acid mixtures enriched with cysteine. Many patients are treated with oral betaine, which provides methyl groups for homocysteine [Hcy] remethylation to Met (Adam et al 2013). Treatment encompassed a combination of diet and betaine in 61 %, betaine alone in 34 % and diet alone in 5 % of 181 European pyridoxine non-responsive CBS patients (Adam et al 2013).

Despite the variability of approaches, treatment effectively reduces morbidity, especially when initiated early. A

significant reduction of vascular morbidity has been observed in 158 treated patients (Yap et al 2001). While 112 vascular complications were predicted on the basis of historical data from untreated CBS deficient patients, only 17 vascular complications occurred in 12 patients (Yap et al 2001). Thirteen CBS patients identified by NBS and with good compliance to treatment had normal full-scale IQ results (mean 105.8) in contrast to six poorly compliant (mean IQ 80.8) and two untreated patients (IQ 52 and 53) (Yap et al 2001). These findings are supported by 11 patients diagnosed on NBS who developed no somatic symptoms and had a normal IQ (Walter et al 1998). In 14 late diagnosed patients, all had lens subluxation/dislocation and impaired vision at diagnosis and eye disease progressed with less strict adherence to treatment. In contrast, 15 early treated patients had no evidence of lens subluxation and better visual acuity (Mulvihill et al 2001).

Recommendation The severe form of CBS deficiency is defined by its significant morbidity (milder forms are also known). A large subgroup of patients is responsive to pyridoxine administration. Low methionine/low protein diet (mostly combined with methionine-free and cysteine enriched amino acid mixture) and/or betaine administration is effective in pyridoxine non-responsive CBS deficiency. Normal cognitive development and prevention of physical

complications is possible in patients detected by NBS and treated early with continuous strict compliance.

Methods for NBS in CBS deficiency

Since the 1970s NBS for CBS deficiency has been based on the detection of increased Met concentration in dried blood spots (DBS) (Chace et al 1996; Mudd 2011). Long-term experience with the NBS for CBS deficiency exists namely in Ireland, Italy, Portugal, and some U.S. states (McHugh et al 2011; Naughten et al 1998; Peterschmitt et al 1999; Accinni et al 2003; Martins et al 2012). Varying proportions (20–50 %) of missed pyridoxine non-responsive cases have been reported with different Met cut-off values used (Naughten et al 1998; Gan-Schreier et al 2010). Lowering of the Met cut-off from 138 to 67 $\mu\text{mol/L}$ doubled the detection rate of CBS deficiency (Peterschmitt et al 1999). Accordingly, Met cut-off values as low as 40 $\mu\text{mol/L}$ have been proposed to yield higher sensitivity (McHugh et al 2011; Turgeon et al 2010). Sensitivity is also increased by using the Met/Phe ratio which adjusts for protein intake (Bowron et al 2005). In conclusion, the primary factor driving sensitivity is the selection of as low as possible cut-off values for Met and Met/Phe ratio.

Although the pyridoxine responsive form of CBS deficiency is common in various populations and represented 44 % of patients in the historical cohort of 629 patients (Mudd et al 1985), only 3–13 % of patients detected by NBS reported in the literature were pyridoxine responders (Mudd et al 1985; Janosík et al 2009). This marked discrepancy indicates that NBS programs using Met as the primary marker most likely fail to detect the majority of patients with pyridoxine responsive disease (Mudd 2011).

Specificity of Met as the primary marker is poor since blood concentrations also increase in other disorders, e.g., liver disease, MATI/III, GNMT and SAHH deficiencies, and tyrosinemia type I (Mudd 2011). Specificity may be improved by quantifying tHcy in DBS as a second-tier marker (Bártl et al 2014; Turgeon et al 2010; Alodaib et al 2012) leading to a fivefold reduction of the false positive rate compared to the period when only Met at a cut-off of 40 $\mu\text{mol/L}$ was used (Bowron et al 2005).

Data on 124 CBS deficient patients ascertained by NBS show wide variation of the Met concentration in DBS with a clearly increased median of 103 $\mu\text{mol/L}$ compared to the median of 20 $\mu\text{mol/L}$ in healthy neonates (Table 3) (McHugh et al 2011). A target cut-off value for Met to differentiate the 99th centile in healthy newborns from the 5th centile in confirmed true positive cases recommended by R4S is in the range between 39 and 50 $\mu\text{mol/L}$. The Met/Phe ratio has slightly better performance characteristics. Data on tHcy in DBS as a secondary marker at the time of screening is available from 31 CBS deficient patients showing a clear distinction between cases (median tHcy 40 $\mu\text{mol/L}$) and controls

(6 $\mu\text{mol/L}$). When tHcy is measured on the same specimen as a second tier test, calculating the Met/tHcy ratio may help differentiate the patients with CBS deficiency from MATI/III deficient individuals (see Table 3).

The measurement of tHcy as primary NBS marker has only been used in the native Qatari population due to technical requirements and high costs (Gan-Schreier et al 2010). Molecular genetic testing is feasible and may be an option for high-risk populations with a limited number of prevalent mutations. However, this approach will miss individuals carrying other mutations (Gan-Schreier et al 2010).

Recommendation NBS for CBS deficiency can be performed by detecting elevated methionine, methionine-to-phenylalanine ratio and/or hyperhomocysteinemia in dried blood spots. However, total homocysteine has only exceptionally been used as a primary marker. Sensitivity of methionine as a primary marker for pyridoxine non-responsive CBS deficiency is limited and inversely related to the chosen cut-off concentrations of methionine. For the pyridoxine responsive form of the disease sensitivity is largely unknown and probably low. Specificity of methionine as a primary marker may be substantially increased by analyzing total homocysteine as a second tier marker and calculating the Met/tHcy ratio.

The combined remethylation defects cblC, cblF, cblJ, and cblD-MMA-Hcy

Clinical findings

The cblC defect, caused by mutations in the *MMACHC* gene is the most common of these defects (Lerner-Ellis et al 2009). Mutations in the *LMBRD1* gene cause the cblF defect (Rutsch et al 2009), and *ABCD4* mutations the cblJ defect (Coelho et al 2012). Depending on type and location of the mutation in the *MMADHC* gene, the cblD defect either presents as isolated methylmalonic acid (MMA)-uria (cblD-MMA, beyond the scope of this report), combined MMA and homocystinuria (cblD-MMA-Hcy, resembling the cblC, cblF and cblJ defects) or isolated homocystinuria (cblD-Hcy, clinically indistinguishable from the cblE or cblG defect, addressed below) (Jusufi et al 2014). All defects affect the synthesis of methylcobalamin, the co-factor for the cytosolic enzyme methionine synthase, and of adenosylcobalamin, the coenzyme for the intramitochondrial enzyme methylmalonyl-CoA mutase, which metabolizes MMA (Fig. 1). Thus low Met, elevated tHcy, propionylcarnitine and MMA are biochemical hallmarks of the disorders (McHugh et al 2011; Fowler 1998).

Main clinical symptoms of the early-onset form of the cblC defect (<12 months, 88 % of cases) (Fischer et al 2014) include failure to thrive, macrocytic anemia, muscular hypotonia, developmental delay, dysmorphic features, thinned corpus callosum, hydrocephalus, hemolytic uremic syndrome,

and decreased visual acuity due to pigmentary retinopathy but no lens dislocation (Rosenblatt et al 1997; Weisfeld-Adams et al 2013; Fischer et al 2014). Late-onset patients may present any time during childhood or adult life, typically with various combinations of myelopathy, dementia, psychiatric symptoms, hemolytic-uremic syndrome, thromboembolism, and pulmonary hypertension; retinopathy occurs rarely (Fischer et al 2014; Huemer et al 2014a).

Treatment of the cblC defect — which has also been applied in cblF, cblJ, and cblD-Hcy-MMA defects — consists of intramuscular hydroxycobalamin (OH-Cbl), oral betaine and folate, rarely combined with Met supplementation. Carnitine is often added but has no proven effect (Martinelli et al 2011). Dosages of medications vary considerably (Fischer et al 2014; Carrillo-Carrasco et al 2012).

In early-onset cblC patients, overall survival, hematological symptoms, and failure to thrive improve while eye disease and cognitive impairment often progress under treatment (Martinelli et al 2011; Fischer et al 2014). Despite identification by NBS and early treatment, 11/12 patients showed developmental delay, 8/9 brain pathologies, 10/11 muscular hypotonia, and 9/12 nystagmus at a mean age of 50 months (Weisfeld-Adams et al 2013). However, from a screened and early treated cohort of 13 patients from Tuscany, the ten patients subjected to psychometric testing achieved a mean DQ of 80 or IQ of 85 compared to a mean DQ/IQ of 45 and 66 in 18 clinically diagnosed patients (mean age at diagnosis: 54 days). Only 7/13 screened and early treated patients developed the characteristic eye disease compared to 11/13 clinically diagnosed early-onset patients (Pasquini 2014, personal communication).

In most late-onset cblC patients, dementia, renal function, myelopathy, and axonal neuropathy improve on treatment. Long-lasting disease before treatment initiation correlates with incomplete recovery and residual pathologies (Huemer et al 2014a).

Clinical information is available for less than twenty patients with the cblF defect. Three patients died at 5, 9, and 10 months, respectively (Shih et al 1989; Bowron et al 2005; Alfadhel et al 2011). In some early treated patients, neurocognitive outcome was good (Miousse et al 2011; Armour et al 2013), while in others severe developmental delay was present (Gailus et al 2010; Alfadhel et al 2011). For the cblJ defect (Coelho et al 2012), clinical information on treatment outcome is extremely limited. Two patients reportedly responded well to treatment with methylcobalamin (Kim et al 2012). In the reported cblD-Hcy-MMA cases, a broad spectrum of responses to treatment has been observed ranging from fatal progression of symptoms (Miousse et al 2009) to improved (Suormala et al 2004; Miousse et al 2009) to even nearly normal development (Suormala et al 2004) (Table 2).

Recommendation Screening for the cblC defect should be considered since in the early-onset CblC defect response to treatment has been observed for non-neurological symptoms with improved overall survival. The impact of early treatment on neurocognitive development and retinopathy is unclear. For late-onset patients treatment is mostly beneficial, especially when initiated before irreversible organ damage has occurred. The present knowledge allows no conclusion concerning the benefit of early treatment for the cblD-Hcy-MMA, cblF and cblJ defects.

Methods for NBS in the combined remethylation defects

Newborn screening for these defects has used the primary markers propionylcarnitine (C3), propionylcarnitine/ acetylcarnitine (C3/C2) ratio, Met, and Met/Phe ratio. The R4S data indicate that the sensitivity of C3/C2 is superior to Met and Met/Phe. Indeed, the use of cut-off values for C3/C2 between 0.18 and 0.20 $\mu\text{mol/L}$ differentiates between the 99th centile in healthy newborns and the 5th centile of cases with cblC, cblD-Hcy-MMA and cblF (McHugh et al 2011). In contrast, both Met and Met/Phe will miss a considerable proportion of cases if used as a primary marker alone (McHugh et al 2011) (Table 3).

The specificity of C3 and/or C3/C2 for detecting these diseases is generally low (la Marca et al 2007; Turgeon et al 2010) since they also detect nutritional imbalances (including maternal vitamin B12 deficiency) or other inborn errors of metabolism (Scolamiero et al 2014). Low Met has been proposed as a possible secondary marker (Weisfeld-Adams et al 2010), but due to the above mentioned shortcomings, other second tier strategies have been developed, applying isolated or simultaneous measurement of MMA, methylcitric acid and tHcy (Alodaib et al 2012; la Marca et al 2007; Tortorelli et al 2010; Fu et al 2013). While methylcitric acid does not differentiate cblC/cblD patients from controls, the positive predictive value substantially increased from 4 to 100 % by measurement of MMA and from 11 to 36 % by measuring tHcy (la Marca et al 2007; Turgeon et al 2010).

Recommendation NBS for the cblC defect can be performed by identification of elevated propionylcarnitine with a high propionylcarnitine/acetylcarnitine ratio. Low methionine or low methionine/phenylalanine ratio are less sensitive. Sensitivity of propionylcarnitine and propionylcarnitine/ acetylcarnitine ratio for detecting mild forms of the cblC defect is unknown. Specificity of biomarkers substantially increases by analyzing methylmalonic acid and/or total homocysteine in DBS as a second tier test. It can be anticipated that the same markers are useful for the detection of cblD-Hcy-MMA, cblF or cblJ disorders but efficacy and feasibility of screening for these three disorders is unknown. It should be

Table 2 Disease course and treatment response in homocystinurias and methylation disorders

Disease	Disease course	Response to treatment
CBS deficiency B ₆ -non responsive	Severe	Early, strict treatment results in favorable physical and cognitive outcome.
CBS deficiency B ₆ -responsive	Mild to asymptomatic	Good response to pyridoxine
cbIC defect	Severe	Early-onset: Survival, hematological and microangiopathy symptoms responsive. Neurological, ophthalmological and developmental problems often ongoing Late-onset: Predominantly responsive, residual myelopathy/neuropathy frequent in prolonged untreated courses
cbID-Hcy defect	Severe	Variable, very limited data
cbID-MMA-Hcy defect		
cbIF defect	Severe	Variable, very limited data
cbIJ defect	Severe	Variable, very limited data
Severe MTHFR deficiency	Severe	Early betaine treatment is beneficial
cbIE and cbIG defect	Severe	Treatment seems beneficial in a majority of patients but response is variable. Individual case reports encourage early treatment
MAT I/III deficiency, heterozygous individuals	Predominantly benign	Generally good without treatment
MAT I/III deficiency, compound heterozygote and homozygote individuals	White matter disease, cognitive impairment observed	Methionine-lowering treatment may be beneficial but data is very limited
GNMT deficiency	Predominantly benign	Generally good without treatment
SAHH deficiency	Severe	Variable, very limited data
ADK deficiency	Severe	Unknown

noted that by using these markers, maternal vitamin B12 deficiency might be detected.

Isolated remethylation defects: the cbIE, cbIG, and cbID-Hcy defects, and severe MTHFR deficiency

Clinical findings

The cbIG defect affects the enzyme methionine synthase (MTR) while methionine synthase reductase (MTRR) is defective in the cbIE defect (Watkins and Rosenblatt 2012). The clinically similar cbID-Hcy defect was discussed above. All defects impair the remethylation of Hcy to Met and result in elevated tHcy and low normal to low Met (Stabler et al 2013; Huemer et al 2014b).

The clinical pattern of the cbIE and the cbIG defect is indistinguishable. Mostly, initial symptoms evolve in the first year of life or in early childhood (Zavadakova et al 2005). Macrocytic and/or megaloblastic anemia, failure to thrive and feeding difficulties, cognitive dysfunction, nystagmus, impaired visual acuity but no lens dislocation, and seizures are frequent. Microangiopathy and hemolytic uremic syndrome have only very rarely been described (Huemer et al 2014b). A subgroup of three patients from the Iberian Peninsula presented with isolated macrocytic anemia (Vilaseca et al 2003).

Treatment encompasses folic or folinic acid, betaine and a variety of cobalamin (OH-Cbl, cyano- or methylcobalamin) preparations. Mostly tHcy and Met as well as macrocytic anemia (Fowler et al 1997; Vilaseca et al 2003) and neurocognitive performance (Fowler et al 1997; Harding et al 1997; Vilaseca et al 2003; Müller et al 2007; Schiff et al 2011) respond well to treatment. In four patients, a positive impact of early treatment on outcome has been observed (Müller et al 2007; Schiff et al 2011). However, in a series of nine cbIE patients, the overall impact of treatment on neurodevelopmental disabilities and eye disease was “at most moderate” (Zavadakova et al 2005). Eye disease seems not to respond to treatment (Huemer et al 2014b).

Severe methylenetetrahydrofolate reductase (MTHFR) deficiency also results in defective methylation of Hcy to Met. The enzyme MTHFR catalyzes the reduction of 5,10 methylenetetrahydrofolate to 5-methyltetrahydrofolate, which serves as a methyl donor for the methylation of Hcy to Met (Fattal-Valevski et al 2000). In addition to elevated tHcy and decreased Met the folate concentrations in plasma and especially in CSF are decreased in severe MTHFR deficiency (Thomas and Rosenblatt 2005; Stabler et al 2013). As in the combined defects, due to the enhanced flux through the transsulfuration pathway, plasma cystathionine (median 1192 nmol/L) is grossly elevated in patients compared to controls (median 157 nmol/L) (Stabler et al 2013).

Table 3 Laboratory markers for newborn screening of homocystinurias and methylation defects in R4S

Disease	Marker	Healthy newborns-concentration in DBS					Patients-concentration in DBS					Recommended range for cut-off value	Comment
		Number of laboratories with data	1 %	median	99 %	Number of patients	1 %	5 %	median	90 %	99 %		
CBS deficiency	Met ($\mu\text{mol/L}$)	154	10.65	20	39	124	26	51	103	540	964	>39–50	
	Met/Phe	92	0.23	0.41	0.75	107	0.55	0.97	2.17	11.50	25	>0.75–0.97	
	tHcy ($\mu\text{mol/L}$)	3	4.12	6.00	10.70	31	12.46	14.2	40	80	158	>0.7–12.4	
	Low Met/tHcy	2	2.24	5.10	12.08	29	0.86	0.93	2.24	4.29	5.75	<4.00–5.00	
	C3 acylcarnitine ($\mu\text{mol/L}$)	151	0.58	1.68	4.3	198	2.65	3.46	8.40	13.3	19	>4.3–5.4	poor sensitivity
	C3/C2 acylcarnitine	136	0.036	0.08	0.18	187	0.13	0.20	0.39	0.62	1.67	>0.18–0.20	
	MMA ($\mu\text{mol/L}$)	3	0.010	0.20	1.52	28	7.13	10.94	28	114	437	>3.0–7.0	
	Methylcitrate ($\mu\text{mol/L}$)	2	0.036	0.21	12.4	10	0.37	0.38	0.63	6.13	10.42	>0.7–1.2	
	Met ($\mu\text{mol/L}$)	154	10.65	20	39	178	3.62	4.90	10.2	26	47	<10.2–10.6	poor sensitivity
	Low Met/Phe	92	0.23	0.41	0.75	23	0.050	0.081	0.18	0.47	0.70	<0.17–0.23	poor sensitivity
cbIF	tHcy ($\mu\text{mol/L}$)	3	4.12	6.00	10.70	23	14.66	20	52.00	172	241	>10.7–12.4	
	C3 acylcarnitine ($\mu\text{mol/L}$)	151	0.58	1.68	4.3	4	8.75	8.88	10.18	12.87	13.76	>4.3–5.4	
	Low Met ($\mu\text{mol/L}$)	154	10.65	20	39	17	2.52	3.78	5.91	9.76	10.22	<10.2–10.6	
	Low Met/Phe	92	0.23	0.41	0.75	17	0.038	0.068	0.10	0.16	0.17	<0.17–0.23	
	tHcy ($\mu\text{mol/L}$)	3	4.12	6.00	10.70	8	42	48	70	132	154	>10.7–12.4	
	Low Met/tHcy	2	2.24	5.10	12.08	8	0.034	0.048	0.083	0.17	0.25	<4.00–5.00	
	Met ($\mu\text{mol/L}$)	154	10.65	20	39	143	44	50	103	173	582	>39–50	
	Met/Phe	92	0.23	0.41	0.75	137	0.84	1.1	1.77	3.72	6.32	>0.75–0.97	
	Met/tHcy	2	2.24	5.10	12.08	2	10.87	12.53	31	48	52	>14.00–15.00	
	cbE/cbIG/ MTHFR	Low Met ($\mu\text{mol/L}$)	154	10.65	20	39	17	2.52	3.78	5.91	9.76	10.22	<10.2–10.6

In severe MTHFR deficiency, developmental delay, seizures, and movement disorders are frequent in children. Adolescents and adults may present with psychiatric symptoms, neuropathy or thromboembolic events. Retinopathy has only rarely been reported (Thomas and Rosenblatt 2005) but data from a mouse model show impaired retinal function (Lawrance et al 2011). Megaloblastic and/or macrocytic anemia is usually absent (Thomas and Rosenblatt 2005). Treatment with folate, folinic acid, Met, pyridoxine, and different cobalamin preparations has generally been considered unsuccessful (Thomas and Rosenblatt 2005). However, Met supplementation improved the course in an early onset case (Abeling et al 1999). In a systematic review including 36 patients, the positive impact of early betaine treatment on outcome in early-onset patients was shown (Diekman et al 2014). Five early treated patients survived with normal psychomotor development while nine of ten non-treated and two late treated patients died. In families with one or more deceased siblings, none of the treated but all untreated children died. Psychomotor development was impaired in all children with delayed treatment onset, despite stabilization from introduction of treatment (Diekman et al 2014) (Table 2).

Recommendation NBS for severe MTHFR deficiency is recommended since early betaine administration is associated with a positive outcome. NBS for the cblD-Hcy, cblE, and cblG defects can be considered since in the majority of clinically diagnosed patients, treatment seems beneficial and there is some evidence for an added benefit of early treatment.

Methods for NBS in the cblD-Hcy, cblE, and cblG defects, and severe MTHFR deficiency

Neonatal screening for these disorders became possible only after the introduction of tandem mass spectrometry for determination of decreased Met concentration in DBS (Chace et al 1996). Data on 18 remethylation cases were available in R4S (four MTHFR, one cblG, and one cblE and 12 non-classified cases). The Met concentration in the DBS was decreased to a median of 5.91 $\mu\text{mol/L}$ in the patients, with no overlap with healthy newborns (Table 3). In addition, both the Met/Phe ratio (Bowron et al 2005) and the second-tier marker tHcy clearly differentiate patients from controls (Tortorelli et al 2010; McHugh et al 2011).

In theory, tHcy can be employed as the primary screening marker but there are no published data on its use. There are no long-term data on efficacy of screening programs for these disorders (Tortorelli et al 2010) owing to their rarity and low proportion of screening programs addressing decreased Met or Met/Phe ratio (in R4S, 162 laboratories have listed a high cut-off for methionine, but only 73 have a low cut-off value).

Recommendation Neonatal screening for the cblD-Hcy, cblE, and cblG defects, and for MTHFR deficiency appears to be possible by detecting a decreased methionine and methionine-to-phenylalanine ratio in DBS followed by analysis of total homocysteine as a second tier marker. Efficacy and feasibility of screening for these disorders is largely unknown.

Methionine adenosyltransferase deficiency

Clinical findings

Methionine adenosyltransferase (MAT) catalyses the formation of S-adenosylmethionine (AdoMet) from Met. Three variant forms (MAT I, II and III) of the enzyme have been described. The MAT I and III — encoded by a single gene (*MAT1A*) — are expressed mainly in liver. The MATII is expressed ubiquitously in extrahepatic tissues and seems to play a pivotal role in maintaining whole body AdoMet homeostasis. Homozygosity or compound heterozygosity for *MAT1A* mutations and heterozygosity for some mutations result in hypermethioninemia without elevation of AdoMet concentrations. Inheritance of this biochemical trait may thus be autosomal recessive or autosomal dominant (Baric 2009).

There is evidence that a small number of individuals homozygous or compound heterozygous for *MAT1A* mutations develop myelination defects or white matter disease and some of these patients are cognitively impaired. These clinical signs have been related to persistent very high Met concentrations and/or to AdoMet deficiency in the brain (17, 63). It is advisable to monitor these patients closely and to consider introduction of a Met restricted diet or AdoMet supplementation. The available evidence suggests that AdoMet supplementation may normalize existing white matter changes (Surtees et al. 1991).

However, the most common autosomal dominant form of hypermethioninemia detected by neonatal screening is caused by heterozygosity for the mutation c. 791G>A (p.R264H). Since the clinical course in these individuals is generally benign (Baric 2009; Mudd 2011), the impact of a protein-controlled diet cannot clearly be evaluated (Couce et al 2013) and the usefulness of early detection of these individuals is questionable (Mudd 2011; Blom 2014, personal communication) (Table 2).

Recommendation In individuals heterozygous for MAT I/III deficiency, treatment seems not to be necessary. Patients homozygous/compound heterozygous for MAT I/III mutations should be monitored closely and may require intervention. Data on the benefit of early intervention is lacking.

Methods for NBS in MATI/III

Most MAT I/III deficient individuals were originally identified by NBS programs targeting CBS deficiency (Mudd 2011) and the number of detected individuals substantially increased with use of lower Met cut-off values between 45 and 54 $\mu\text{mol/L}$ (Martins et al 2012). The incidence is especially high in the Galicia region of Spain and in Portugal (1:26,000–1:28,163), mostly due to heterozygosity for the p.R264H mutation (Martins et al 2012; Couce et al 2013). The DBS concentrations of Met in 143 MAT I/III deficient individuals (including carriers) in the R4S database reached a median of 103 $\mu\text{mol/L}$ compared to the median of 20 $\mu\text{mol/L}$ in healthy neonates (McHugh et al 2011). A target cut-off value for Met to differentiate between the 99th centile in healthy newborns from the 5th centile in confirmed true positive cases is consequently in the range between 39 and 50 $\mu\text{mol/L}$ (Table 3). The Met/Phe ratio has similar performance characteristics (McHugh et al 2011). Differentiation between MATI/III and CBS deficiency based on Met concentrations is not possible. However, determination of tHcy concentration as second tier strategy and then calculating the Met/tHcy ratio may discriminate between CBS and MATI/III deficiency; a simple and effective approach is to apply the dual scatter plot functionality available on the website of the R4S collaborative project (Table 3).

Recommendation Individuals with MATI/III deficiency have been predominantly detected in NBS programs for CBS deficiency due to increased methionine concentrations. The majority of these newborns are heterozygotes for the mutation c.791G>A and the benefit of detecting these individuals is questionable. At present, there is no primary marker to differentiate between MATI/III and CBS deficiency. Data on the Met/tHcy ratio to differentiate between these two conditions are promising but limited.

Glycine-N-methyltransferase deficiency,
S-adenosylhomocysteine hydrolase deficiency and adenosine kinase deficiency

Another disorder characterized by isolated hypermethioninemia is glycine-N-methyltransferase (GNMT) deficiency, which has only been described in three individuals. GNMT transfers a methyl group from S-adenosylmethionine to glycine and thus synthesizes sarcosine. Deficiency of the enzyme results in gross elevation of AdoMet and Met concentrations. GNMT deficiency seems to be a relatively benign disorder presenting with mild hepatomegaly or moderately elevated liver transaminases (Baric 2009). At present, the rarity and the benign course of the condition do not support inclusion in NBS programmes (Mudd 2011).

The enzyme S-adenosylhomocysteine hydrolase (SAHH) catalyses the hydrolysis of S-adenosylhomocysteine to Hcy and adenosine. SAHH deficiency has been described in seven patients (Honzík et al 2012). Biochemical findings include grossly increased S-adenosylhomocysteine with elevation of S-adenosylmethionine and Met while tHcy may be normal or mildly elevated. The clinical course in the first male index patient from Croatia revealed a severe disease with myelination disorder and low IQ, hepatopathy and myopathy with elevated creatine kinase. The pathogenesis of myopathy in SAHH deficiency is at present not completely understood. While Met restriction had a positive impact on neurocognitive symptoms even in the index patient and improved the course significantly in the patient's two early diagnosed and treated brothers, myopathy persisted in all cases (Barić et al 2005; Baric 2009). In another patient, treatment from the age of 5 months to 5 years failed to prevent clinical symptoms (Baric 2009; Mudd 2011). Recently two siblings with hydrops fetalis and fatal outcome have been reported (Grubbs et al 2010), underlining the severity of the disease.

The enzyme ADK catalyzes the phosphorylation of adenosine, which is produced by hydrolysis of S-adenosylhomocysteine by SAHH. When adenosine is not further metabolized, its accumulation inhibits S-adenosylhomocysteine hydrolase leading also to an increase of the upstream metabolites S-adenosylmethionine and methionine (Bjursell et al 2011). Until now adenosine kinase (ADK) deficiency has been described in six patients: two Swedish siblings and two related pairs of Malaysian siblings with severe developmental delay, macrocephaly, epilepsy, and abnormal liver function. One patient died at the age of 10 years. All patients shared the biochemical pattern of elevated methionine, S-adenosylmethionine, and S-adenosylhomocysteine levels in the presence of normal/slightly elevated tHcy similar to SAHH deficiency. The detection of increased urinary adenosine may be useful to differentiate this defect from SAHH deficiency, in which elevated creatine kinase activity can also be expected. Methionine restriction may be beneficial in some patients (Mudd 2011). Patients may be identified by NBS programs based on elevated Met (Buist et al 2006), however no targeted NBS approaches for SAHH and ADK deficiencies have been reported so far.

Recommendation GNMT appears to be a benign condition. In the very small numbers of patients described, SAHH and ADK deficiency are predominantly severe disorders. Present knowledge allows no conclusive statement concerning the benefit of early treatment for SAHH and ADK deficiency. Neonatal screening for SAHH and ADK deficiency cannot be recommended at this stage due to lack of data and validated screening strategies.

Conclusion

Selection of diseases for NBS must be driven by clinical arguments — a severe disease, generally not detectable early in life on a clinical basis, feasible and effective treatment, and a better outcome in early treated individuals meets accepted criteria for inclusion into a NBS program (Wilson and Jungner 1968). However, the evidence for efficacy of treatment in the homocystinurias and methylation disorders is limited due to small series of subjects with different genotypes, varying treatment, and inconsistent follow-up strategies. Particularly, time of initiation of treatment and the use, dosage, and intervals of application of drugs are far from uniform.

However, despite the above-mentioned limitations of clinical data, there is robust evidence for successful treatment and improved outcome by early treatment for CBS deficiency, and to a lesser extent for MTHFR deficiency and the cblC defect. In CBS deficiency, good outcome in individuals identified by NBS is consistently reported even from various centers including patients with a variety of CBS mutations and despite a large variability in treatment practice (Naughten et al 1998; Walter et al 1998). In MTHFR deficiency, the clear clinical effect of early treatment with betaine is an important supporting evidence for screening (Diekman et al 2014). In the late-onset cblC defect, treatment response is seen in almost all patients but residual disease manifestations are frequent in patients with long duration of clinical abnormalities before treatment initiation (Huemer et al 2014a). Evidence for efficacy of early treatment is less clear for the early-onset cblC defect in which survival, hematological symptoms, and feeding problems often improve, while the effect on neurocognitive development and retinopathy is less encouraging (Weisfeld-Adams et al 2013).

If screening for these disorders is advisable from a clinical point of view, robust, applicable, and widely accepted primary and secondary markers must be used. The respective markers and ratios should be developed and their utility tested by combining a large amount of data and by application of post-analytical interpretative tools (Marquardt et al 2012; Hall et al 2014). Algorithms and cut-offs for markers based on small isolated case series have a significant risk of bias and may be misleading. However, even with the use of large sample numbers the selection of reliable cut-off values for NBS based on comparing data from patients detected by the NBS methods and values from healthy newborns is not trivial. Very little data is available on missed cases and milder forms of the screened diseases, which may manifest years or even decades later. Therefore, it must be kept in mind that NBS markers reflect the present state of the art, which is temporary and may change in the future due to technological advances and improved knowledge.

For the other disorders addressed by this review, there is either only weak evidence for a response to treatment and for

the specific value of early treatment [cblE and cblG defects] (Vilaseca et al 2003; Zavadakova et al 2005), or insufficient data to evaluate treatment outcome [cblD, cblF, cblJ, GNMT, SAHH, and ADK deficiency]. It must be emphasized that NBS programs targeting CBS, MTHFR or cblC will most probably identify also subjects with other homocystinurias/methylation disorders with uncertain clinical relevance and that biochemical algorithms to differentiate between all these disorders must be established. On one hand, this will allow a more differentiated view regarding the benefit of early detection and treatment for these disorders and facilitate genetic counselling for families with severely affected children. On the other hand, subjects requiring no treatment and follow-up may also be detected. A paradigm for this process is the MAT I/III defect for which data on clinical severity are conflicting. Heterozygosity for specific *MAT1A* mutations is considered benign and these individuals should not but will be picked up. However, a small but significant number of individuals homozygous or compound heterozygous for *MAT1A* mutations may develop clinical complications and may benefit from treatment.

To improve the overall knowledge on homocystinurias and methylation disorders, studies on the natural course, treatment, and outcome following a structured scheme are needed. The E-HOD registry is collecting data on a large number of patients to create a large, standardized database and thereby facilitate and support considerations on whether to recommend inclusion or exclusion of these disorders into/from NBS panels.

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Conflict of Interest None.

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