EPIDEMIOLOGY OF RARE DISEASES DETECTED BY NEWBORN SCREENING IN THE CZECH REPUBLIC

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SUMMARY

Objectives: Presymptomatic detection of patients with rare diseases (RD), defined by a population frequency less than 1 : 2,000, is the task of newborn screening (NBS). In the Czech Republic (CZ), currently eighteen RD are screened: phenylketonuria/hyperphenylalaninemia (PKU/HPA), congenital hypothyroidism (CH), congenital adrenal hyperplasia (CAH), cystic fibrosis (CF), medium chain acyl-CoA dehydrogenase deficiency (MCADD), long chain 3-hydroxyacyl-CoA dehydrogenase deficiency (LCHADD), very long chain acyl-CoA dehydrogenase deficiency (VLCADD), carnitine palmitoyl transferase I and II deficiency (CPTID, CPTIID), carnitine-acylcarnitine translocase deficiency (CACTD), maple syrup urine disease (MSUD), glutaric aciduria type I (GA I), isovaleryl-CoA dehydrogenase deficiency (IVA), argininemia (ARG), citrullinemia (CIT), biotinidase deficiency (BTD), cystathionine beta-synthase-deficient homocystinuria (CBSD HCU), and methylenetetrahydrofolate reductase deficiency homocystinuria (MTHFRD HCU). The aim was to analyze the prevalence of RD screened by NBS in CZ.

Methods: We examined the NBS programme in CZ from 1 January 2010 to 31 December 2017, which covered 888,891 neonates. Dried blood spots were primarily analyzed using fluorescence immuno-assay, tandem mass spectrometry and fluorimetry.

Results: The overall prevalence of RD among the neonate cohort was 1 : 1,043. Individually, 1 : 2,877 for CH, 1 : 5,521 for PKU/HPA, 1 : 6,536 for CF (1 : 5,887 including false negative patients), 1 : 12,520 for CAH, 1 : 22,222 for MCADD, 1 : 80,808 for LCHADD, 1 : 177,778 for GA I, 1 : 177,778 for IVA, 1 : 222,223 for VLCADD, 1 : 296,297 for MSUD, 1 : 8,638 for BTD, and 1 : 181,396 for CBSD HCU.

Conclusions: The observed prevalence of RD, based on NBS, corresponds to that expected, more precisely it was higher for BTD and lower for MSUD, IVA, CBSD HCU, MCADD and VLCADD. Early detection of rare diseases by means of NBS is an effective secondary prevention tool.

Key words: rare disease, newborn screening, Czech Republic, public health, epidemiology, prevention

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INTRODUCTION

Rare diseases (RD) are defined by a population frequency less than 1 : 2,000 and represent a heterogeneous group of up to 8,000 disorders (1). Despite significant therapeutic advances in the treatment of RD, many patients still suffer from insufficient diagnostics and inadequate care. With a view toward standardizing and harmonizing evidence-based health practices in the European Union, the Committee of Experts on RD (EUROCERD*) was established in 2010 (2). The Government of the Czech Republic (CZ), through Resolution No. 466 of 14 June 2010, endorsed the "National Strategy for Rare Diseases for the Years 2010–2020" (3), which summarizes the issue of rare diseases from both the European Union and CZ viewpoints and proposes a core set of objectives and measures to improve RD diagnosis and treatment in CZ. This Resolution includes newborn screening (NBS) as an important area since all diseases included in NBS are classified as RD and NBS represents a model approach to RD diagnosis and treatment.

NBS is an effective secondary prevention tool (4), for active population-wide detection of congenital and/or inherited

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diseases or defects in the early preclinical stages (5). The greater the number of neonates in a region that are screened, the greater the effectiveness of the NBS system. NBS is based on specific substances concentration measurement in dried blood spots (DBS) on filter paper and in selected probands on pathogenic allelic variants analysis in the same DBS. The overall NBS system is comprised of a reliable pre-analytical process (standardization of sample collection, timing, repeated samples, etc.), an analytical process (selection of laboratory methods, storage and utilization of samples), and a post-analytical process (protocols or procedures used in positive or unclear findings) (6). The rules of all these activities are summarized in the Methodological Guidelines Manual of the Czech Ministry of Health, which defines the medical lege artis procedures in the NBS including methods of diagnostic confirmation and subsequent care of patients detected by NBS (7).

NBS was first implemented in the CZ in 1975, for phenylketonuria/hyperphenylalaninemia (PKU/HPA) (8). NBS then expanded, in 1985 it was used for congenital hypothyroidism (CH) (9), in 2006 for 21-hydroxylase deficiency (congenital adrenal hyperplasia, CAH) (10), in 2009 for cystic fibrosis (CF) (11), and nine other inherited metabolic disorders (IMD), i.e., medium chain acyl-CoA dehydrogenase deficiency (MCADD), long chain 3-hydroxyacyl-CoA dehydrogenase deficiency (LCHADD), very long chain acyl-CoA dehydrogenase deficiency (VLCADD), carnitine palmitoyl transferase I and II deficiency (CPTID, CPTIID), carnitine-acylcarnitine translocase deficiency (CACTD), maple syrup urine disease (MSUD), glutaric aciduria, type I (GA I), and isovaleryl-CoA dehydrogenase deficiency (IVA) (12). The latest expansion was made in June 2016, when five additional IMD were added, i.e., argininemia (ARG), citrullinemia (CIT), biotinidase deficiency (BTD), cystathionine beta-synthase-deficient homocystinuria (CBSD HCU), and methylenetetrahydrofolate reductase deficiency homocystinuria (MTHFRD HCU).

The aim of this study was to analyze the epidemiology of RD screened by NBS in the CZ between years 2010 and 2017.

MATERIALS AND METHODS

The study (from 1 January 2010 to 31 December 2017) was based on results derived from DBS on filter paper that were collected from the heel pricks and sent to specified laboratories by mail (Table 1). DBS were taken between the 48th–72th hour of newborn's life. Our epidemiological data show the prevalence of screened RD at the time of DBS sampling.

The analysis included 888,891 neonates, which covers 100% of the Czech neonatal population (2010–2017). Data were collected from newborn screening laboratories (Table 1). DBS were tested for:

- thyroid-stimulating hormone (TSH), using fluorescence immuno-assay (FIA; Delfia a AutoDelfia produced by Perkin-Elmer) for detection of CH (13);
- 17-hydroxyprogesterone (17-OHP) for detection of CAH using the above-mentioned method (10);
- immunoreactive trypsinogen (IRT) for detection of CF using FIA, and from the group with the highest IRT levels, a subsequent DNA analysis of the *CFTR* (cystic fibrosis transmembrane conductance regulator) gene was performed (initially 32 and from July 2010, 50 common variants were tested using the commercial Elucigene assays produced by Elucigene Diagnostics) using the original DBS (11);
- amino acids and acylcarnitines for detection of IMD were determined using tandem mass spectrometry (MS/MS) using kits (MassChrom Reagent produced by Chromsystems) and MS/MS instrumentation (API 2000TM, API 3200TM and API 4000TM produced by AB Sciex), namely PKU/HPA, MCADD, LCHADD, VLCADD, CPTID, CPTIID, CACTD, MSUD, GA I, IVA, ARG, CIT, CBSD HCU, and MTHFRD HCU (14). Patients with BTD were detected using fluorimetry.

Newborns with positive NBS findings were referred for follow-up to appropriate clinical centres to confirm the diagnosis using generally accepted diagnostic standards: in cases with a confirmed diagnose patients started subsequent care (7). Decision limits for the screened disorders are summarized in Table 2 and confirmatory test criteria in Table 3 (15–19). The numbers of confirmed diagnoses from NBS were based on feedback reports from clinical care centres. In cases with unclear results (i.e. between negative and positive decision limits), disease specific protocols were applied (mostly repeated DBS sampling). The percentage of newborns with a final negative result was presented as the false positive rate (FPR). FPR was calculated as the ratio between the number of false positives and the total number of negatives findings. Positive predictive value (PPV) stated the probability that newborns with a positive screening test truly had the disease (the percentage of patients with a positive test who actually had the disease). PPV was calculated as the ratio between the number of true positives and the number of true positives and false positives findings).

RESULTS

The overall prevalence of RD among the neonate cohort was 1:1,043. Individually, 1 : 2,877 for CH, 1 : 5,521 for PKU/HPA, 1 : 6,536 for CF (1 : 5,887 including false negative patients), 1 : 12,520 for CAH, 1 : 22,222 for MCADD, 1 : 80,808 for LCHADD, 1 : 177,778 for GA I, 1 : 177,778 for IVA, 1 : 222,223 for VLCADD, 1 : 296,297 for MSUD, 1 : 8,638 for BTD, and 1 : 181,396 for CBSD HCU. Table 4 shows results of NBS in CZ from 2010–2017.

Table 1. *Newborn screening laboratories in the Czech Republic*

Immunoanalytical methods	Tandem mass spectrometry, fluorimetry	DNA analysis
Department of Children and Adolescents, University Hospital Královské Vinohrady, Prague	Department of Paediatrics and Adolescent Medicine, General University Hospital, Prague	Department of Biology and Medical Genetics, University Hospital Motol, Prague
Department of Clinical Biochemistry, University Hospital Brno	Department of Clinical Biochemistry, University Hospital Olomouc	Department of Medical Genetics, University Hospital Brno

Table 2. *Decision (positivity) limits for analytes detected in dried blood spots*

Disorder	Analyte	Decision limit (capillary blood)
Congenital hypothyroidism	Thyroid-stimulating hormone (TSH)	TSH ≥ 15.0 mIU/L
Cystic fibrosis	Immunoreactive trypsinogen (IRT) and *CFTR* (cystic fibrosis transmembrane conductance regulator) gene mutations	IRT > 99.0 percentile (65.0 ng/mL) and *CFTR* mutation on at least one allele or without mutation and IRT ≥ 200 ng/mL
Congenital adrenal hyperplasia (21-hydroxylase deficiency)	17-hydroxyprogesterone (17-OHP)	17-OHP according to birthweight/gestational age, range 20.0–160 nmol/L, example: 20.0 nmol/L for ≥ 2700 g (≥ 37 gestational week)
Phenylketonuria/hyperphenylalaninemia	Phenylalanine (Phe) Tyrosine (Tyr)	Phe > 120 µmol/L and Phe/Tyr ratio > 2.00
Medium chain acyl-CoA dehydrogenase deficiency	Octanoylcarnitine (C8) Acetylcarnitine (C2)	C8 > 0.40 (0.50) µmol/L and C8/C2 ratio > 0.02 (0.03) µmol/L
Long chain 3-hydroxyacyl-CoA dehydrogenase deficiency	Hydroxypalmitoylcarnitine (C16OH) Hydroxyoleoylcarnitine (C18:1OH)	C16OH > 0.10 µmol/L or C18:1OH > 0.10 (0.07) µmol/L
Very long chain acyl-CoA dehydrogenase deficiency	Tetradecenoylcarnitine (C14:1) Acetylcarnitine (C2) Palmitoylcarnitine (C16)	C14:1 > 0.55 (0.40) µmol/L and C14:1/C2 ratio > 0.03 and C14:1/C16 ratio > 0.26 (0.15)
Carnitine palmitoyl transferase I deficiency	Free carnitine (C0) Palmitoylcarnitine (C16) Oleoylcarnitine (C18:1) Acetylcarnitine (C2)	C0 > 60.3 (57.0) µmol/L and C0/(C16+C18) ratio > 25.0 (29.0) and (C16+C18:1)/C2 ratio < 0.10
Carnitine palmitoyl transferase II deficiency Carnitine-acylcarnitine translocase deficiency	Palmitoylcarnitine (C16) Oleoylcarnitine (C18:1) Acetylcarnitine (C2)	C16 > 5.06 (7.00) µmol/L and (C16+C18:1)/C2 ratio > 0.35 (0.48)
Glutaric aciduria type I	Glutarylcarnitine (G5DC) Octanoylcarnitine (C8) Palmitoylcarnitine (C16)	C5DC > 0.40 (0.60) µmol/L and C5DC/C8 ratio > 5.40 (C5DC/C16 > 0.40)
Maple syrup urine disease	Leucine (Leu) Isoleucine (Isoleu) Hydroxyproline (Hyp) Alanine (Ala) Valine (Val) Tyrosine (Tyr) Phenylalanine (Phe)	Leu + Isoleu + Hyp > 270 µmol/L (Leu > 260 µmol/L) and Leu/Ala ratio > 1.40 (1.25) or Leu + Val/Phe + Tyr > 3.79
Isovaleryl-CoA dehydrogenase deficiency	Isovalerylmethybutyrylcarnitine (C5) Free carnitine (C0) Propionylcarnitine (C3) Octanoylcarnitine (C8)	C5 > 1.00 (0.60) µmol/L and C5/C0 ratio > 0.03 and C5/C3 ratio > 0.39 (C5/C8 ratio > 20.0)
Citrullinemia	Citrulline (Cit) Ornithine (Orn) Phenylalanine (Phe)	Cit > 70.0 (56.0) µmol/L and Orn/Cit ratio < 2.09 (2.51) and Cit/Phe ratio > 0.95 (0.81)
Biotinidase deficiency	Biotinidase serum activity	Biotinidase serum activity < 30.0% than median of health population
Cystathionine beta-synthase-deficient homocystinuria	Methionine (Met) Phenylalanine (Phe) Homocystein (Hcys)	Met > 33.0 (36.4) µmol/L and Met/Phe ratio > 0.58 (0.41) and Hcys > 12.0 (15.0) µmol/L
Methylenetetrahydrofolate reductase deficiency homocystinuria	Methionine (Met) Phenylalanine (Phe) Homocystein (Hcys)	Met < 7.00 µmol/L or Met/Phe ratio < 0.15 (0.10) and Hcys > 12.00 (15.0) µmol/L
Argininemia	Arginine (Arg) Ornithine (Orn) Phenylalanine (Phe)	Arg > 60.0 (63.0) µmol/L and Arg/Orn ratio > 0.75 (0.40) and Arg/Phe ratio > 1.02 (0.98)

Decision limits for amino acids and acylcarnitines in parentheses are for non-derivatized assays.

Table 3. *Confirmatory test criteria for screened disorders*

Disorder	Definition (venous blood sample)
Congenital hypothyroidism	Thyroid-stimulating hormone (TSH) > 8.00 mIU/L or free thyroxine (fT4) < 12.0 pmol/L
Cystic fibrosis	Sweat test ≥ 60.0 mmol/L or 30.0–59.0 mmol/L and two pathogenic mutations in cystic fibrosis transmembrane conductance regulator (*CFTR*) gene
Congenital adrenal hyperplasia (21-hydroxylase deficiency)	Basal level of 17-hydroxyprogesterone (17-OHP) above reference range and/or positive cosyntropin test and casual mutation in *CYP21A2* gene
Phenylketonuria/hyperphenylalaninemia	Phenylalanine (Phe) > 120 μmol/L
Medium chain acyl-CoA dehydrogenase (MCAD) deficiency	Octanoylcarnitine (C8) and acetylcarnitine (C2) above reference range, and MCAD deficiency or two pathogenic mutations in *ACADM* gene or decreased fatty acid oxidation (FAO) in lymphocytes
Long chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD) deficiency	Hydroxypalmitoylcarnitine (C16OH) and hydroxyoleoylcarnitine (C18:1OH) above reference range, and LCHAD deficiency or two pathogenic mutations in *HADHA* gene or decreased FAO in lymphocytes
Very long chain acyl-CoA dehydrogenase (VLCAD) deficiency	Tetradecenoylcarnitine (C14:1) and acylcarnitine (C2) and palmitoylcarnitine (C16) above reference range and VLCAD deficiency or two pathogenic mutations in *ACADVL* gene or decreased FAO in lymphocytes
Carnitine palmitoyl transferase (CPT) I deficiency	Free carnitine (C0) and palmitoylcarnitine (C16) and oleoylcarnitine (C18:1) and acetylcarnitine (C2) above reference range and CPT I deficiency or two pathogenic mutations in *CPT IA* gene or decreased FAO in lymphocytes
Carnitine palmitoyl transferase (CPT) II deficiency	Palmitoylcarnitine (C16) and oleoylcarnitine (C18:1) and acetylcarnitine (C2) above reference range, and CPT II deficiency or two pathogenic mutations in *CPT2* gene
Carnitine-acylcarnitine translocase (CACT) deficiency	Palmitoylcarnitine (C16) and oleoylcarnitine (C18:1) and acetylcarnitine (C2) above reference range and CACT deficiency or two pathogenic mutations in *SLC25A20* gene
Glutaric aciduria type I	Glutarylcarnitine (C5DC) and octanoylcarnitine (C8) and palmitoylcarnitine (C16) above reference range and glutaryl CoA dehydrogenase deficiency or two pathogenic mutations in *GCD* gene
Maple syrup urine disease	Leucine (Leu) and isoleucine (Isoleu) and hydroxyproline and alanine (Ala) and valine (Val) and tyrosine (Tyr) and phenylalanine (Phe) above reference range and branched-chain ketoacid dehydrogenase (BCKAD) deficiency or two pathogenic mutations in *BCKDHA* gene or *BCKDHB* gene or *DBT* gene
Isovaleryl-CoA dehydrogenase deficiency	Isovalerylmethybutyrylcarnitine (C5) and free carnitine (C0) and propionylcarnitine (C3) and octanoylcarnitine (C8) above reference range and isovaleryl CoA dehydrogenase deficiency or two pathogenic mutations in *IVD* gene
Citrullinemia	Argininosuccinate synthase deficiency or two pathogenic mutations in *ASS1* gene
Biotinidase deficiency	Biotinidase deficiency or two pathogenic mutations in *BTD* gene
Cystathionine beta-synthase-deficient homocystinuria	Cystathionine beta-synthase deficiency or two pathogenic mutations in *CBS* gene
Methylenetetrahydrofolate reductase deficiency homocystinuria	Methylenetetrahydrofolate reductase deficiency or two pathogenic mutations in *MTHFR* gene
Argininemia	Arginase deficiency or two pathogenic mutations in *ARG1* gene

DISCUSSION

We have quantified prevalence at the age of DBS sampling. Considering that screened RD are inherited conditions and thanks to NBS can be treated in a timely manner, and based on a normal life expectancy during childhood, it can be assumed that the prevalence of RD is almost the same as their frequency and incidence in the general paediatric population. Table 5 summarizes the data from the literature of other countries relative to the prevalence of RD screened in CZ (18, 20–22). A comparison with our results leads us to conclude that the prevalence in the Czech population is higher for BTD, but lower for MSUD, IVA, CBSD HCU, MCADD, and VLCADD. However, in case of low prevalence, the statistical effect of small numbers may occur.

One interesting result was the markedly lower population frequency of CF than reported previously, i.e. 1 : 2,700 based on clinical observations in CZ (23). The explanation for this difference likely rests with the increasing effect of prenatal diagnosis and better-informed reproductive decisions (22).

The cause of false negativity in CF NBS is predominantly due to lower IRT levels in CF newborns with meconium ileus (6 cases from 15 false negative patients). While these infants can be detected clinically, there are nevertheless a small number of CF newborns that escape detection due to very rare mutations on both alleles.

The improving efficacy of NBS to detect RD in CZ was also documented by the increasing cumulative screening prevalence with the stepwise expansion of screened disorders from 1 : 2,701

Table 4. *Results of newborn screening in the Czech Republic from 2010–2017 (N = 888,891)*

Disease	Period	Number of screened neonates (n)	Number of confirmed diagnosis (n)	Screening prevalence	Number of FP (n)	FPR total (%)	PPV
PKU/HPA	Jan 1, 2010–Dec 31, 2017	888,891	161	1 : 5,521	238	0.0268	0.40
CH			309	1 : 2,877	197	0.0222	0.61
CAH			71	1 : 12,520	3696	0.4158	0.02
CF			136	1 : 6,536	967	0.1088	0.12
MCADD			40	1 : 22,222	17	0.0019	0.70
LCHADD			11	1 : 80,808	4	0.0004	0.73
VLCADD			4	1 : 222,223	62	0.0070	0.06
CPTID			0	–	29	0.0033	–
CPTIID/CACTD			0	–	2	0.0002	–
MSUD			3	1 : 296,297	90	0.0101	0.03
GA I			5	1 : 177,778	29	0.0033	0.15
IVA			5	1 : 177,778	75	0.0084	0.06
ARG	Jun 1, 2016–Dec 31, 2017	181,396	0	–	1	0.0006	–
CIT			0	–	10	0.0055	–
BTD			21	1 : 8,638	34	0.0187	0.38
CBSD HCU			1	1 : 181,396	10	0.0055	0.09
MTHFRD HCU			0	–	3	0.0017	–
Total			767	1 : 1,043	5,464	0.6387	0.12

ARG – argininemia; BTD – biotinidase deficiency; CACTD – carnitine-acylcarnitine translocase deficiency; CAH – congenital adrenal hyperplasia; CBSD HCU – cystathionine beta-synthase-deficient homocystinuria; CF – cystic fibrosis; CH – congenital hypothyroidism; CIT – citrullinemia; CPTID – carnitine palmitoyl transferase I deficiency; CPTIID – carnitine palmitoyl transferase II deficiency; FP – false positivity; FPR – false positive rate; GA I – glutaric aciduria type I (glutaryl-CoA dehydrogenase deficiency); IVA – isovaleryl-CoA dehydrogenase deficiency (isovaleric acidaemia); LCHADD – long chain 3-hydroxyacyl-CoA dehydrogenase deficiency; MCADD – medium chain acyl-CoA dehydrogenase deficiency; MSUD – maple syrup urine disease; MTHFRD HCU – methylenetetrahydrofolate reductase deficiency homocystinuria; PKU/HPA – phenylketonuria/hyperphenylalaninemia; PPV – positive predictive value; VLCADD – very long chain acyl-CoA dehydrogenase deficiency

Table 5. *Literary data on prevalence of screened rare diseases (18, 20–22)*

Disease	Prevalence
Congenital hypothyroidism	1 : 2,600
Phenylketonuria/hyperphenylalaninemia	1 : 2,000–1 : 10,000
Cystic fibrosis	1 : 3,000–1 : 13,500
Congenital adrenal hyperplasia	1 : 14,000
Medium chain acyl-CoA dehydrogenase deficiency	1 : 15,000
Cystathionine beta-synthase-deficient homocystinuria	1 : 60,000
Biotinidase deficiency	1 : 30,000–1 : 60,000
Citrullinemia	1 : 40,000
Long chain 3-hydroxyacyl-CoA dehydrogenase deficiency	1 : 100,000
Isovaleryl-CoA dehydrogenase deficiency (isovaleric acidaemia)	1 : 100,000
Maple syrup urine disease	1 : 150,000
Very long chain acyl-CoA dehydrogenase deficiency	1 : 11,000–1 : 100,000
Carnitine palmitoyl transferase II deficiency	1 : 100,000
Argininemia	1 : 1,000,000
Carnitine palmitoyl transferase I deficiency	1 : 1,000,000
Carnitine-acylcarnitine translocase deficiency	1 : 1,000,000
Glutaric aciduria type I (glutaryl-CoA dehydrogenase deficiency)	Unknown
Methylenetetrahydrofolate reductase deficiency homocystinuria	Unknown

between 2002–2005 to 1 : 2,072 in 2007–2008 (24), to 1 : 1,043 in the currently evaluated period (2010–2017). In a comparison of the number of screened disorders in Europe (25), CZ ranks better than average. On the other hand, the achieved high NBS detection levels were associated with an increasing frequency of repeated DBS, with the current cumulative FPR 0.64%. FPR impacts the healthy population and can stigmatize neonates and their families (26). Reducing the FPR is a challenge and one of the objectives of the NBS system. From our results, CAH has the highest FPR, with other screened disorders having significantly lower FPR. An effective way to reduce FPR is by implementation of a secondary analytic tier based on the original DBS: in case of CAH for example, using liquid chromatography with MS/MS (27). Pilot studies looking for ways to address this issue are in progress abroad and in CZ.

The greater number of diseases screened by the NBS system corresponds with technological progress and analytic potential. However, expanding the NBS system can create problems, not only technical, but also ethical, economic, legislative, and political ones. Current Czech legislation does not allow the nationwide NBS, which would be primarily based on genome analysis, e.g. NBS for spinal muscular atrophy, although it would be effective for early diagnosis and therapy (28).

The above-mentioned issues have led to discussions about adding or refining the original criteria of the NBS system, defined in 1968 by Wilson and Jungner (5). Every NBS expansion is associated with questions about the selection criteria. Traditional screening criteria can function as guidelines even if their universal applicability has been questioned by new biotechnologies and scientific progress. Before adding a new disorder to the screening panel, it is necessary to evaluate the balance between health benefits and potential harms (26). In 2010–2011, the European Network of Experts on Newborn Screening (EUNENBS) created a questionnaire study and published a list of 26 diseases, which could be included in European NBS system (29). The list is divided into basic groups with higher and lower prevalence and candidate groups (Table 6). In CZ, the study of NBS of severe combined immunodeficiency (SCID) has already been methodologically prepared (30).

Table 6. *Recommendation of diseases for newborn screening in Europe (29)*

Basic group		Candidate group
Diseases with higher prevalence	**Diseases with lower prevalence**	
PKU/HPA, CH, CAH, CF, MCADD, Th	MSUD, GA I, GAL	BD, CPTIID, CACTD, GA II, HMGD, HCSD, HCU, IVA, BKT, LCHADD, LSD, 3MCC, TYR I, TYR II, TYR III, VLCADD, vitamin B12 deficiency, SCID, CMV

3MCC − 3-methylcrotonyl-CoA carboxylase deficiency; BD − biotinidase deficiency; BKT − β-ketothiolase deficiency; CACTD − carnitine-acylcarnitine translocase deficiency; CAH − congenital adrenal hyperplasia; CF − cystic fibrosis; CH − congenital hypothyroidism; CMV − congenital cytomegalovirus infection; CPTIID − carnitine palmitoyl transferase II deficiency; HCU − homocystinuria; HCSD − holocarboxylase synthetase deficiency; HMGD − HMG-CoA lyase deficiency; GA I, II − glutaric aciduria type I, II; GAL − galactosemia; IVA − isovaleryl-CoA dehydrogenase deficiency; LCHADD − long chain 3-hydroxyacyl-CoA dehydrogenase deficiency; LSD − lysosomal storage disorders; MCADD − medium chain acyl-CoA dehydrogenase deficiency; MSUD − maple syrup urine disease; PKU/HPA − phenylketonuria/hyperphenylalaninemia; SCID − severe combined immunodeficiency; Th − thalassemia; TYR I, II, III − tyrosinemia type I, II, III; VLCADD − very long chain acyl-CoA dehydrogenase deficiency

CONCLUSIONS

The prevalence of screened RD in the Czech population mostly corresponds with internationally published data, actually, it was found higher for BTD and lower for MSUD, IVA, CBSD HCU, MCADD, and VLCADD.

NBS in CZ detects patients with RD in the early preclinical stages and the level of NBS corresponds with the standard used by many states of the European Union. NBS in CZ represents an efficient tool to improve the quality of care for patients with RD. The next important steps in NBS optimization will be to examine additional analytical methods to reduce false positivity, consider expanding the list of screened disorders and discuss decision limits which can detect milder forms, e.g. in CH.

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Conflict of Interests

None declared

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