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ABSTRACT

INTRODUCTION: Newborn screening is a public health programme for early diagnosis of treatable diseases.

METHODS: The subjects included were newborns born 2002-2019. Expanded newborn screening (eNBS) for metabolic diseases was introduced as a pilot project from 2002 to 2009, followed by routine screening with informed dissent. A total of 967,780 newborns were screened; 82,930 were unscreened. Furthermore, a historic cohort of clinically diagnosed children born in the 1992-2001 period was included. Children in the unscreened and historic cohorts were evaluated for the same diseases as were the screened children. Dried blood spot samples were collected locally and sent for screening analyses. We recorded newborns with true and false positive results as well as false negative results and their clinical signs at screening and at the last follow-up.

RESULTS: A total of 603 samples were screen positive: 354 false positives and 249 true positives (222 newborns and 27 mothers). The positive predictive value (PPV) was 41% for the entire screening period; 62% for 2018. The false positive rate (FPR) was 0.036% overall; 0.024% for 2018. The overall prevalence of diseases was 1:3,900; in the historic cohort, the prevalence of the same diseases was 1:8,300; 7.3% had symptoms at the time of screening. At follow-up, 93% of the children had no clinically significant sequelae. Among 82,930 unscreened newborns, 27 (1:3,000) had eNBS panel diseases, some with severe manifestations.

CONCLUSIONS: This update of eNBS in Denmark confirms that eNBS is a successful preventive public health programme. Early treatment in a latent phase of disease is effective and screening should be extended to other diseases not currently in the programme.

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TRIAL REGISTRATION: not relevant.

Newborn screening (NBS) is a public health programme for early diagnosis of treatable, mostly genetic, diseases, such as inherited metabolic diseases (IEM). Early treatment in a latent disease stage can prevent disease manifestations, which for diseases on many NBS panels include irreversible brain and organ damage and death [1-3]. Internationally, NBS was started > 50 years ago and in Denmark for phenylketonuria (PKU) in 1975. NBS for PKU became the paradigm for a well-functioning, cost-effective screening. It enabled expansion of the disorders screened for: in Denmark, hypothyroidism was added in 1977 and expanded newborn screening (eNBS) was added in 2002. Today, the panel includes 17 diseases (**Table 1**); see also **Figure 1** for a Danish NBS timeline. Some countries screen for > 60 diseases, type and number varying, depending on demography and differences in how to select diseases for NBS [1, 4]. However, most refer to the Wilson and Jungner criteria (**Table 2**) [5]. This manuscript focuses on eNBS for IEM in Denmark using mostly tandem mass spectrometry and presents current experiences after screening of nearly a million newborns.

METHODS

We included all children born from 1 February 2002 to 12 February 2019 [6]. The period included a pilot period until 2 February 2009, with eNBS done following written informed consent, followed by eNBS done in a routine NBS programme with informed dissent, i.e. active withdrawal from screening after information was provided (Figure 1). The percentage screened changed during the period from 65% at the start to 85% at the end of the pilot period and during the routine period 99.85%. A total of 967,780 newborns were screened with eNBS in the full period with 82,930 (8.6%) remaining unscreened.

We included a historic cohort of children born from 1 January 1992 to 31 December 2001, diagnosed clinically with a disease from the eNBS panel.

The diseases included in the screening panel changed during the reported period as shown in Figure 1 and **Table 3**. Decision on the current panel was based on results from the pilot period and a review including scoring of the screening potential for selected diseases [7]. All diseases with a scoring > the 75th centile (and

ORIGINAL ARTICLE

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TABLE 1 / Current Danish newborn screening panel[a].

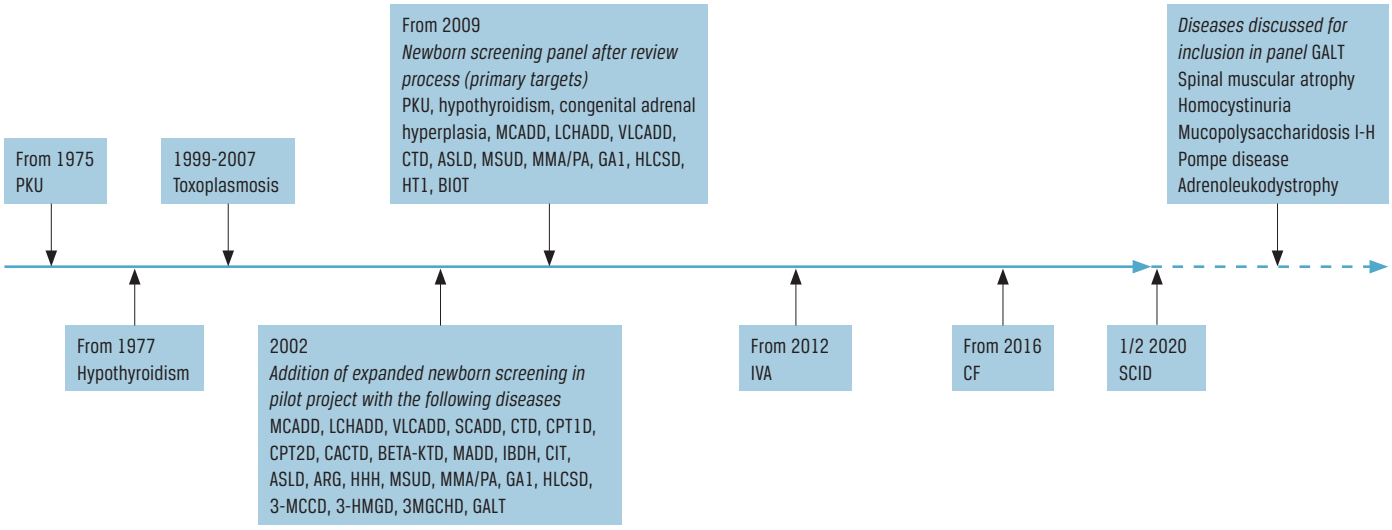
Targets	Disease
Primary	
Fatty acid oxidation diseases	Medium-chain acyl-CoA dehydrogenase deficiency
	Very long-chain acyl-CoA dehydrogenase deficiency
	Long-chain 3-hydroxy acyl-CoA dehydrogenase deficiency incl. trifunctional protein deficiency
	Carnitine transporter deficiency
Aminoacidopathies	Classical phenylketonuria
	Tyrosinaemia type 1
	Argininosuccinic acidaemia
	Classical maple syrup urine disease
Organic acidemias	Methylmalonic acidaemia incl. cobalamin A + B diseases
	Propionic acidaemia
	Isovaleric acidaemia
	Glutaric acidaemia type 1
	Holocarboxylase synthetase deficiency
	Biotinidase deficiency, profound and partial
Other	Congenital hypothyroidism
	Congenital adrenal hyperplasia
	Cystic fibrosis
	Severe combined immunodeficiency[b]
Secondary	Biopterin cofactor deficiencies
	Mild hyperphenylalaninaemia
	Medium/-short-chain L-3-hydroxy acyl-CoA dehydrogenase deficiency
	Multiple acyl-CoA dehydrogenase deficiency
New, discussed for adding to panel	Classical galactosaemia, homocystinuria, spinal muscular atrophy, mucopolysaccharidosis type 1, Pompe disease, adrenoleukodystrophy

IEM = inborn error of metabolism; MS = mass spectrometry; NBS = newborn screening; SSI = Statens Serum Institut.
a) Routine newborn screening panel in Denmark. The NBS is state-operated, free of charge, done in an informed dissent set-up and covers Denmark, The Faroe Islands and Greenland corresponding to 62,000 births per year. Blood sampling is done 48-72 hours postpartum via heelprick and subsequent spotting on filter paper blood spot cards. Primary NBS analyses are done in a single centralised laboratory (Statens Serum Institut (SSI)). Confirmatory testing is done in the Centre for Inherited Metabolic Diseases, Copenhagen and Research Unit for Molecular Medicine, Aarhus. Spare blood spots have been stored in the Danish Newborn Screening Biobank at the SSI, Copenhagen, since 1982. All children with a true-positive screening result for an IEM are managed in the Centre for Inherited Metabolic Diseases, Copenhagen. Review of new screening targets and application to the Danish Health Authority for inclusion in the routine panel as well as supervision of performance and quality assurance of The NBS are done by The Committee for Clinical Genetics and Screening, Danish Paediatric Society and the Danish Tandem MS Working Group. The systematic review process of Danish NBS that lays the ground for the current Danish NBS practice is described in a report from the Danish Health Authority, 2008 – please contact the authors for further information.
b) Coming soon.

ABBREVIATIONS

3-HMGD = 3-hydroxy-3-methyl-glutaryl-CoA lyase deficiency (OMIM ID: 246450)
3-MCCD = 3-methylcrotonyl-CoA-carboxylase deficiency (OMIM IDs: 210200, 210210)
3-MGCHD = 3-methylglutaconyl-CoA hydratase deficiency (OMIM ID: 250950)
ARG = hyperargininemia due to arginase deficiency (OMIM ID: 207800)
ASLD = argininosuccinate lyase deficiency (OMIM ID: 207900)
BETA-KTD = beta-ketothiolase deficiency (OMIM ID: 203750)
BIOTD = biotinidase deficiency (OMIM ID: 253260)
CACTD = carnitine/acylcarnitine translocase deficiency (OMIM ID: 212138)
CAH = congenital adrenal hyperplasia (OMIM ID: 201910)
CH = congenital hypothyroidism
CIMD = Center of Inherited Metabolic Diseases
CIT = citrullinaemia type 1 due to arginosuccinate synthase deficiency (OMIM ID: 215700)
CPT1D = carnitine palmitoyl transferase 1 deficiency (OMIM ID: 600528)
CPT2D = carnitine palmitoyl transferase 2 deficiency (OMIM IDs: 255110, 600649, 608836)
CTD = carnitine transporter deficiency (OMIM ID: 600528)
eNBS = expanded newborn screening
FAOD = fatty acid oxidation disease
FPR = false positive rate
GA1 = glutaric aciduria type 1 due to glutaryl-CoA dehydrogenase deficiency (OMIM ID: 231670)
GALT = galactosaemia due to galactose 1-phosphate uridyl transferase deficiency (OMIM ID: 230400)
IEM = inborn error of metabolism
HHH = hyperornithinaemia, hyperammonaemia, homocitrullinuria due to ornithine translocase deficiency (OMIM ID: 238970)
HLCSD = holocarboxylase synthetase deficiency (OMIM ID: 253270)
HT1 = hepatorenal tyrosinaemia (type 1) due to fumarylacetoacetase deficiency (OMIM ID: 276700)
IVA = isovaleric acidaemia due to isovaleryl-CoA dehydrogenase deficiency (OMIM ID: 243500)
LCHADD = long-chain 3-hydroxy acyl-CoA dehydrogenase deficiency (OMIM ID 609016)
MADD = multiple acyl-CoA dehydrogenase deficiency (OMIM ID: 231680)
MCADD = medium-chain acyl-CoA dehydrogenase deficiency (OMIM ID: 201450)
MMA = methylmalonic aciduria (many OMIM IDs, most relevant here are: 251000, 251100, 251110)
MS/MS = tandem mass spectrometry
MSUD = classic maple syrup urine disease due to branched-chain alfa-keto acid dehydrogenase deficiency (OMIM ID: 248600)
NBS = newborn screening
PA = propionic acidaemia due to propionyl-CoA carboxylase deficiency (OMIM ID: 606054)
PKU = classical phenylketonuria due to phenylalanine hydroxylase deficiency (OMIM ID: 261660)
PPV = positive predictive value
SCADD = short-chain acyl-CoA dehydrogenase deficiency (OMIM ID: 201470)
SSI = Statens Serum Institut
TPD = trifunctional protein deficiency (OMIM ID: 609015)
VLCADD = very long-chain acyl-CoA dehydrogenase deficiency (OMIM ID: 201475)

FIGUR 1 / Timeline for the Danish newborn screening.



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some from the 50-75th centile) were reviewed further and a final list of panel diseases was made, which was effective from February 2009 (Figure 1 and Table 1) [7].

Samples consisted of capillary blood collected at local hospitals by heel prick, spotted on filter paper, dried and sent to the screening laboratory (Statens Serum Institut (SSI)). Samples were taken postpartum on day 4-9 in the pilot period (the time of sampling was used routinely until 2009) and at 48-72 hours during routine eNBS [7]; a result was available 2-6 days after sampling. Filter paper blood samples were stored in the Danish Newborn Screening Biobank, SSI. The Center of Inherited Metabolic Diseases, Copenhagen (CIMD) reported newborns with screen-positive results to the local paediatric department, which admitted the child and obtained samples for confirmatory testing. The analyses used at screening and for confirmatory testing are listed in Table 3. Using this infrastructure, the minimal time from birth to start of treatment was 11 days. However, newborns with convincing results and an acutely presenting disorder, like tyrosinemia, were referred directly to the CIMD once results from the SSI became available, making treatment possible at day five.

True-positive results came from newborns in whom or in whose mothers the suspected disease was diagnosed by confirmatory testing. False-positive results were from newborns in whom the suspected disease was not confirmed in the child or the mother. False-negative results were from children with negative results by eNBS who were born in the screening period and had been diagnosed clinically or otherwise, e.g. by family studies, with a disease in the eNBS panel.

Management of children with true-positive results or from the historic cohort took place at the CIMD, serving all of Denmark, the Faroe Islands, and Greenland, making it likely that most children born in this area and diagnosed with diseases in the screening panel are known to the authors. Evaluation of clinical status at last follow-up aimed at disclosing clinically significant sequelae to the disease (**Table 4**). The clinical evaluation was done longitudinally by the same metabolic physician throughout the period. The evaluation was performed in collaboration with local paediatricians – for further details, see [7].

Screening of the first half million newborns has been published previously, including analytical details [7]. The study was approved by the Ethics Committee (KF 01-152/98).

Trial registration: not relevant.

TABLE 2 / The Wilson & Jungner criteria for screening for disease [5].

The condition should be an important health problem
There should be a treatment for the condition
Facilities for diagnosis and treatment should be available
There should be a latent stage of the disease
There should be a test or examination for the condition
The test should be acceptable to the population
The natural history of the disease should be adequately understood
There should be an agreed policy on whom to treat
The total cost of finding a case should be economically balanced in relation to medical expenditure as a whole
Case-finding should be a continuous process, not just a "once and for all" project

RESULTS

A total of 603 samples from 967,780 newborns were screen positive. Of the 603 samples, 249 were true positives and 354 were false positives (Table 3). Patients with true-positive results included 222 newborns and 27 mothers, corresponding to 1:3,887 screened newborns. Biallelic variants were confirmed in relevant genes in all patients with a true positive result. Most variants were convincingly pathogenic; for MCADD, 20% had an uncertain/mild genotype (like the c.199C > T mutation) [2, 7]; for IVA, the "mild" c.932C > T mutation was found in three of four children; in biotinidase deficiency, 36 of 45 children had the "mild" c.1330 G > C mutation and a partial enzyme deficiency.

Positive predictive value (PPV) calculated for the entire screening period was 41%; 15% at start of eNBS and 62% in 2018. The false positive rate (FPR) was 0.036% overall (1:2,781 screened); 0.05% at the initiation of eNBS; and 0.024% (1:4,166 screened) in 2018. Variations in PPV and FPR were seen for different diseases; e.g., PPV was 100% for LCHADD and 11 % for MMA/PA (Table 3). A total of 16 children had false-negative results, 11 of whom had a fatty acid oxidation disease (FAOD), mostly CTD in the beginning of eNBS screening for which the screening algorithm was adjusted [7].

TABLE 3 / Expanded newborn screening panel of diseases, analyses and number found among inborn error of metabolism diagnoses. The diseases included in current expanded newborn screening and additive diseases screened for in the pilot period and removed from panel in 2009. The primary analytes measured during screening and the confirmatory analyses are shown[a].

			True positives		False positives		False negatives			Among unscreened		With maternal disease	
Disease: additive diseases	Primary MS analyte	Confirmatory analyses	n	prevalence, 1:screened, n (%) [2018]	n	prevalence, 1:screened, n (%) [2018]	n	prevalence, 1:screened, n (%)	PPV[b], % [2018]	n	prevalence, 1:screened, n	n	prevalence, 1:screened, n (%)
Current eNBS panel													
Fatty acid oxidation diseases:													
MCADD	C8	PACYLC, DNA	99		14		3		88	2		1	
LCHADD	C16OH	PACYLC, DNA	5		0		0		100	2		0	
VLCADD	C14:1	PACYLC, DNA	5		15		0		25	0		0	
CTD	CO	PACYLC, DNA	32		114		8		22	14		19	
Aminoacidopathies:													
HT1	SUAC	UMET, PAA, DNA	4		0		0		-	0		0	
ASLD	ASA	UMET, PAA, DNA	4		1		0		80	0		0	
MSUD	XLEU	PAA, DNA	3		52		0		5	0		0	
Organic acidaemias:													
MMA/PA	C3	UMET, PACYLC, PAA, DNA	7		58		2		11	1		0	
GA1	C5DC	ENZ, DNA	11		12		2		48	1		0	
IVA	C5	UMET, PACYLC, PAA, DNA	4		2		0		67	0		0	
HLCSD	C5OH	UMET, PACYLC, DNA	4		0		0		-	2		0	
BIOTD	ENZ	ENZ, DNA	45		14		1		76	0		0	
Secondary findings:													
MADD	C5, C8, C18	UMET, PACYLC, DNA	3		2		0		60	1		0	

CONTINUED »

TABLE 3 CONTINUED /

Disease: additive diseases	Primary MS analyte	Confirmatory analyses	True positives n	True positives prevalence, 1:screened, n (%) [2018]	False positives n	False positives prevalence, 1:screened, n (%) [2018]	False negatives n	False negatives prevalence, 1:screened, n (%)	PPV[b], % [2018]	Among unscreened n	Among unscreened prevalence, 1:screened, n	With maternal disease n	With maternal disease prevalence, 1:screened, n (%)
Diseases included earlier during pilot period (2002-2009)													
SCADD	C4	UMET, PACYLC, DNA	0		1		0		-	1		0	
IBDH	C4	UMET, PACYLC, DNA	1		0		0		-	0		0	
CPT1D	C0	UMET, PACYLC, DNA	1		20		0		5	0		0	
CPT2D/CACTD	C16,18:1	UMET, PACYLC, DNA	0		5		0		-	0		0	
BETA-KTD	C5:1	UMET, PACYLC, DNA	0		0		0		-	0		0	
CIT	Cit	UMET, PAA, DNA	2		11		0		15	1		0	
ARG	Arg	UMET, PAA, DNA	0		0		0		-	0		0	
HHH	Hcit	UMET, PAA, DNA	0		2		0		-	0		0	
3-MCCD	C5OH	UMET, PAA, DNA	16		7		0		-	2		7	
3-HMGD	C5OH	UMET, PAA, DNA	1		0		0		-	0		0	
3-MGCHD	C5OH	UMET, PAA, DNA	1		0		0		-	0		0	
GALT	Hex-1-P	Erythrocyte GAL-1-P, DNA	1		19		0		5	0		0	
Total		-	249	1:3,887 (0.026) [1:2,558 (0.039)]	354	1:2,781 (0.036) [1:4,166 (0.024)]	16	1:50,428 (0.002)	41 [62]	27	1:3,189	27	1:3,584 (0.002)
Decade before eNBS: 1992-2001		-	-	1:8,330	-		-		-	-		-	

3-HMGD = 3-hydroxy-3-methyl-glutaryl-CoA lyase deficiency; 3-MCCD = 3-methylcrotonyl-CoA-carboxylase deficiency; 3-MGCHD = 3-methylglutaconyl-CoA hydratase; Arg = arginine; ASLD = argininosuccinate lyase deficiency; ASA = argininosuccinic acid; BETA-KTD = beta-ketothiolase deficiency; BIOTD = biotinidase deficiency; C0 = free carnitine; CX = specific acylcarnitines; CACTD = carnitine/acylcarnitine translocase deficiency; CIT = citrulline; CPT1D = carnitine palmitoyl transferase 1 deficiency; CPT2D = carnitine palmitoyl transferase 2 deficiency; CTD = carnitine transporter deficiency; DNA = molecular-genetic analyses; eNBS = expanded newborn screening; ENZ = enzymatic analyses; GA1 = glutaric aciduria type 1 due to glutaryl-CoA dehydrogenase deficiency; GAL-1-P = galactose 1-phosphate; GALT = galactosaemia due to GAL-1-P uridyl transferase deficiency; Hcit = homocitrulline; Hex-1-P = hexose-1-phosphate; HHH = hyperornithinaemia, hyperammonaemia, homocitrullinuria due to ornithine translocase deficiency; HLCSD = holocarboxylase synthetase deficiency; HT1 = hepatorenal tyrosinaemia type 1 due to fumarylacetoacetase deficiency; IBDH = isobutyryl-CoA dehydrogenase; IVA = isovaleric acidaemia due to isovaleryl-CoA dehydrogenase deficiency; LCHADD = long-chain 3-hydroxy acyl-CoA dehydrogenase deficiency; MADD = multiple acyl-CoA dehydrogenase deficiency; MCADD = medium-chain acyl-CoA dehydrogenase deficiency; MMA = methylmalonic aciduria; MS = mass spectrometry; MSUD = classic maple syrup urine disease due to branched-chain alfa-keto acid dehydrogenase deficiency; PA = propionic acidaemia due to propionyl-CoA carboxylase deficiency; PAA = analysis of amino acids in plasma; PACYLC = analysis of acylcarnitines and C0 in plasma; PPV = positive predictive value; SCADD = short-chain acyl-CoA dehydrogenase deficiency; SUAC = succinylacetone; UMET = urine metabolic screening for amino acids and organic acids; VLCADD = very long-chain acyl-CoA dehydrogenase deficiency; XLEU = concentration of leucine, isoleucine and allo-isoleucine.

a) See [7] for further analytical details.

b) Only calculated for n ≥ 5 and not for the C5OH marker.

The overall prevalence of the diseases included in the programme was 1:3,900. In the decade preceding eNBS, the prevalence for the same diseases was 1:8,300. The increase in prevalence was uneven between diagnoses: MCADD had a prevalence of 1:9,875 compared with 1:35,513 in the decade before eNBS [2, 7]; VLCADD, 3-MCCD, GA1, BIOTD and ASLD had less pronounced increases, while the prevalence of LCHADD, MMA/PA and HT1 was unchanged.

Eighteen of the 222 affected newborns (7.3%) had symptoms at the time of screening, of whom two died (MCADD and MADD), and one had a cardiac arrest with full recovery (MCADD) (Table 4); all newborns with MSUD and citrullinemia and 50% with MMA/PA were symptomatic. On follow-up, 16 children (6.5%) had symptoms related to the disease. Thus, at the last follow-up, 93% of children had no clinically significant sequelae (except for biochemical abnormalities and infrequent rhabdomyolysis and asymptomatic retinopathy in LCHADD). Most children with FAOD, BIOTD, ASLD and HT1 are healthy. A significant percentage of patients with MMA/PA (n = 3/7, 40%) and GA1 (n = 3/11, 30%) developed complications (Table 4).

Among the 82,930 non-screened newborns, 27 had eNBS panel diseases (Table 3). Of these, two had MCADD, both presenting in metabolic crises; two had

TABLE 4 / Symptoms at the time of screening and at the last follow-up: diseases included in current expanded newborn screening and additive diseases screened for in the pilot period and removed from panel in 2009.

Diseases: additive diseases[b]	With symptoms at screening		With symptoms at last follow-up[a]	
	n (%)	clinical findings	n (%)	clinical findings
Current eNBS panel				
Fatty acid oxidation disorders:				
MCADD	5	Cardiac arrest, death, hypoglycaemia, thrombosis	3	Epilepsy, eating disorder, learning difficulties: mild
LCHADD	0	-	0[c]	-
VLCADD	1	Hypoglycaemia	1	Severe rhabdomyolysis
CTD	0	-	1	Cardiomyopathy
Aminoacidopathies:				
HT1	0	-	0	-
ASLD	0	-	0	-
MSUD	3	Metabolic decompensation	2	Developmental delay: moderate
Organic acidurias:				
MMA/PA	3	Metabolic decompensation	3	Developmental delay: mild, renal failure
GA1	0	-	3	Mild dystonia only in 2 children, autism spectrum disorder and developmental delay in 1: moderate
HLCSD	1	Eczema	0	-
BIOTD	0	-	0	-
Secondary findings				
MADD	1	Death	0	-
Diseases included earlier during pilot period				
SCADD	0	-	0	-
CPT1D	1	Hypoglycaemia	0	-
CPT2D/CACTD	0	-	0	-
BETA-KTD	0	-	0	-
CIT	2	Hyperammonaemia	2	Death, liver transplantation
ARG	0	-	0	-
HHH	0	-	0	-
3-MCCD	0	-	0	-
3-HMGD	0	-	0	-
3-MGCHD	0	-	0	-
GALT	1	Liver insufficiency	1	Developmental delay: moderate
Total	18 (7.3)		16 (6.5)	

3-HMGD = 3-hydroxy-3-methyl-glutaryl-CoA lyase deficiency; 3-MCCD = 3-methylcrotonyl-CoA-carboxylase deficiency; 3-MGCHD = 3-methylglutaconyl-CoA hydratase; Arg = arginine; ASLD = argininosuccinate lyase deficiency; BETA-KTD = beta-ketothiolase deficiency; BIOTD = biotinidase deficiency; CACTD = carnitine/acylcarnitine translocase deficiency; CIT = citrulline; CPT1D = carnitine palmitoyl transferase 1 deficiency; CPT2D = carnitine palmitoyl transferase 2 deficiency; CTD = carnitine transporter deficiency; eNBS = expanded newborn screening; GA1 = glutaric aciduria type 1 due to glutaryl-CoA dehydrogenase deficiency; GALT = galactosaemia due to galactose 1-phosphate uridyl transferase deficiency; HHH = hyperornithinaemia, hyperammonaemia, homocitrullinuria due to ornithine translocase deficiency; HLCSD = holocarboxylase synthetase deficiency; HT1 = hepatorenal tyrosinaemia type 1 due to fumarylacetoacetase deficiency; LCHADD = long-chain 3-hydroxy acyl-CoA dehydrogenase deficiency; MADD = multiple acyl-CoA dehydrogenase deficiency; MCADD = medium-chain acyl-CoA dehydrogenase deficiency; MMA = methylmalonic aciduria; MS = mass spectrometry; MSUD = classic maple syrup urine disease due to branched-chain alfa-keto acid dehydrogenase deficiency; PA = propionic acidaemia due to propionyl-CoA carboxylase deficiency; SCADD = short-chain acyl-CoA dehydrogenase deficiency; VLCADD = very long-chain acyl-CoA dehydrogenase deficiency.
a) Follow-up is from few months to 17 years.
b) Abbreviated.
c) < 1 episode of rhabdomyolysis/yr and asymptomatic retinopathy was not included.

LCHADD, one of whom died, and one had severe cardiomyopathy; one GA1 child had a dystonic crisis and one PA child a metabolic decompensation, both with subsequent permanent brain damage.

A maternal IEM was found in 27 cases, dominated by 19 with CTD and seven with 3-MCC. All mothers were asymptomatic.

A total of 29 children with an elevated marker for 3-MCCD (C5OH) were not reported after 3-MCCD was removed from the panel in 2009 (3-MCCD is a non-disease in > 90% of children). However, C5OH is also a marker for holocarboxylase synthase deficiency (HLCSD), common in the Faroe Islands, and a good screening target. Our strategy is to sequence the HLCSD gene in all newborns with a raised C5OH and only report those with two pathogenic mutations in that gene. One of the 29 non-reported children has presented clinically in a 3-MCCD metabolic crisis.

DISCUSSION

The overall frequency of the metabolic diseases included in eNBS was 1:3,900, which represents a 50% increase compared with the frequency during the decade before eNBS. This is similar in other eNBS programmes [8-11]. We believe that the frequencies observed are accurate as all children in Denmark with an IEM are diagnosed and treated in the same centre. For MCADD, a more than three-fold higher incidence after onset of screening was found [2, 7]. VLCADD, 3-MCCD, CTD, BIOTD and ASLD are also found more frequently by screening than by clinical presentation. A proportion of patients may remain asymptomatic throughout life, as illustrated by the 27 asymptomatic mothers with CTD, 3-MCCD or MCADD. On the other hand, many patients with the same diseases will develop symptoms, and the low frequency in the period before eNBS may, in part, be explained by early death or incomplete diagnostic evaluation. Some children died before screening results became available, and these might have remained undiagnosed in the pre-screening era. For MCADD, IVA and BIOTD, mutations associated with mild disease could be documented as one reason for the increased frequency. The incidences of LCHADD, MMA/PA and HT1 were similar to those in the pre-screening era, probably due to a high clinical penetrance.

The higher frequencies of "mild" genotypes in screened as opposed to unscreened patients and the unclear natural histories for some diseases complicate assessment of eNBS benefits [12]. The benefit is most evident for MCADD with a 1% mortality and 97% living healthy lives at the last follow-up (Table 4). This is far better than historic studies with mortality rates of 25% and neurologic sequelae in 25% [13]. Evidence has been presented for GA1 that eNBS markedly improves prognosis [3]; 73% of the children with GA1 in our cohort were clinically normal. Moreover, of the three children with symptoms, two had very mild dystonia only and no history of precipitating metabolic crises. All children with BIOTD, LCHADD or ASLD had no sequelae. In the entire group of children with true-positive results, three (1.2%) died (two before screening results became available), and 93% were without sequelae at the last follow-up. In the cohort of unscreened children, one with LCHADD died; two with MCADD and one with LCHADD had severe metabolic crises; and one with GA1 and one with PA had metabolic crises with subsequent brain damage. Compared with the course of screened children, the course of disease of these six children supports the belief that eNBS is associated with a lower morbidity than diagnosis after clinical presentation. In a study from Boston, 2% of 189 children found by eNBS and 42% of 142 children diagnosed clinically had severe outcomes [14]. Though the precise extent of improved outcome offered by eNBS cannot be reliably evaluated at present, data certainly seem to indicate a substantial clinical benefit.

eNBS diagnosed 27 mothers with 3-MCCD, CTD or MCADD, which is well known from the literature [8, 15]. All mothers with 3-MCCD or CTD were asymptomatic; 3-MCCD has now been removed from the panel, and the clinical significance of CTD in asymptomatic mothers has been questioned [16]. However, symptoms, including cardiac ones, in mothers with CTD found by screening have been observed [15]. These data and sudden death in previously asymptomatic adult patients with CTD [17] argue for continued screening. The high frequency of IEM in mothers of newborns with positive results by eNBS underscores that follow-up investigations should be done of mothers of newborns that were screen positive for these disorders.

During the Danish routine eNBS, FPR was low and PPV high, except in a short period following the introduction of earlier sampling from 2009. This may be explained by an increase in the finding of heterozygotes for FAOD. CTD had the highest FPR. CTD is common in the Faroes Islands [18], and taken together with a high false-negative rate, we had to accept a high FPR. Now a Faroese second screening at 3-4 weeks postpartum is done, enabling a lower FPR. The maternal use of carnitine-depleting pivalic acid-containing antibiotics also contributed to the high FPR.

Confirmation of a flagged result as false or true positive was done within five days after information of the family. Although short, this period is stressful for the family. International data for MCADD indicate that use of health services in families with false-positive results exceeds predicted use during the first year of life [19]. We have no indication of this occurring in Denmark, but it should be studied systematically. Information given in an adequate and sensitive manner is key to reducing anxiety. Apart from a folder about NBS, in Denmark this information is given by the local paediatric department via information sheets from the CIMD, and this seems to function well.

The fact that 99.85% of Danish newborns are screened documents parental trust in NBS and the adequacy of the informed dissent procedure. Few are not screened: very few parents actively decline; some children have died early; some families may not have understood procedures because of language barriers. The frequency of diseases within the eNBS panel was 28 times higher among non-ethnic Danes [20], underscoring the need for thorough information to all ethnic groups.

This up-date on eNBS in Denmark corroborates our first study [7]. Along with international data, it con-

firms that eNBS is a successful preventive public health programme. Early treatment in a latent disease phase is effective, and NBS should be extended to other diseases not currently in the panel. Selection of new diseases is not easy. We removed some diseases from our initial panel because of their high FPR, new evidence of a benign natural history, or presentation before screening. Limited knowledge about natural history, spectrum of severity and effect of treatment is inherent in rare diseases, and evidence about benefit may be obtained only during prospective screening, making gradual adjustments to the NBS programme necessary. Focus should be on treatable diseases; a process of gradual evaluation of screening performance; and relevant psychological and social support to the families, including an acceptance of support also regarding preventive measures in an asymptomatic child.

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