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Biochemical screening of 504,049 newborns in Denmark, the Faroe Islands and Greenland — Experience and development of a routine program for expanded newborn screening

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ABSTRACT

Expanded newborn screening for selected inborn errors of metabolism (IEM) in Denmark, the Faroe Islands and Greenland was introduced in 2002. We now present clinical, biochemical, and statistical results of expanded screening (excluding PKU) of 504,049 newborns during nine years as well as diagnoses and clinical findings in 82,930 unscreened newborns born in the same period. The frequencies of diagnoses made within the panel of disorders screened for are compared with the frequencies of the disorders in the decade preceding expanded newborn screening. The expanded screening was performed as a pilot study during the first seven years, and the experience obtained during these years was used in the development of the routine neonatal screening program introduced in 2009. Methods for screening included tandem mass spectrometry and an assay for determination of biotinidase activity.

A total of 310 samples from 504,049 newborns gave positive screening results. Of the 310 results, 114 were true positive, including results from 12 newborns in which the disease in question was subsequently diagnosed in their mothers. Thus, the overall frequency of an IEM in the screening panel was 1:4942 (mothers excluded) or 1:4421 (mothers included). The false positive rate was 0.038% and positive predictive value 37%. Overall specificity was 99.99%. All patients with true positive results were followed in The Center for

Abbreviations: 3-HMGD, 3-hydroxy-3-methyl-glutaryl-CoA lyase deficiency (OMIM ID: 246450); 3-MCCD, 3-methylcrotonyl-CoA-dehydrogenase deficiency (OMIM IDs: 210200, 210210); 3-MGCHD, 3-methylglutaconyl-CoA hydratase deficiency (OMIM ID: 250950); ACMG, American College of Medical Genetics and Genomics; ARG, hyperargininemia (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; CIT, citrullinemia (OMIM ID: 215700); CPT1D, carnitine palmitoyl transferase 1 deficiency (OMIM ID: 600528); CPT2D, carnitine palmitoyl transferase 2 deficiency (OMIM IDs: 255110, 600649, 608836); CT, congenital toxoplasmosis; CTD, carnitine transporter deficiency (OMIM ID: 600528); DNA, molecular-genetic analyses; ENZ, enzymatic analyses; FN, false negative; FP, false positive; FPR, false positive rate; GA1, glutaric aciduria type 1 (OMIM ID: 231670); GALT, galactosemia (OMIM ID: 230400); GCMS, gas chromatography/mass spectrometry; IEM, inborn error of metabolism; HHH, hyperornithinemia, hyperammonemia, homocitrullinuria (OMIM ID: 238970); HLCSD, holocarboxylase synthetase deficiency (OMIM ID: 253270); HPLC-FL, high performance liquid chromatography with fluorescence detection; HT1, tyrosinemia type 1 (OMIM ID: 276700); IVA, isovaleric aciduria (OMIM ID: 243500); LCHADD, long chain 3-hydroxy acyl-CoA dehydrogenase deficiency (OMIM ID 609016); MADD, multiple acyl-CoA dehydrogenation 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, maple syrup urine disease (OMIM ID: 248600); NA, not applicable; PA, propionic aciduria (OMIM ID: 606054); PAA, plasma amino acids; PACYLC, plasma acylcarnitines; PKU, classical phenylketonuria (OMIM ID: 261660); PPV, positive predictive value; SCADD, short chain acyl-CoA dehydrogenase deficiency (OMIM ID: 201470); TP, true positive; TPD, trifunctional protein deficiency (OMIM ID: 609015); UPLC-MS/MS, ultra performance liquid chromatography tandem mass spectrometry; UPLC-UV, ultra performance liquid chromatography with UV-spectrophotometric detection; U, arbitrary units; UAA, urine amino acids; UOA, urine organic acids; VLCADD, very long chain acyl-CoA dehydrogenase deficiency (OMIM ID: 201475).

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Inherited Metabolic Disorders in Copenhagen, and the mean follow-up period was 45 months (range 2-109 months). There were no deaths among the 102 children, and 94% had no clinically significant sequelae at last follow-up.

Our study confirms the higher frequency of selected IEM after implementation of expanded newborn screening and suggests an improved outcome for several disorders. We argue that newborn screening for these disorders should be standard of care, though unresolved issues remain, e.g. about newborns with a potential for remaining asymptomatic throughout life. Well organized logistics of the screening program from screening laboratory to centralized, clinical management is important.

1. Introduction

Screening newborns using tandem mass spectrometry (MS/MS) allows for simultaneous detection of multiple metabolites in one integrated analysis, and thereby for simultaneous screening for many inherited metabolic disorders. MS/MS and other methods, like assay of biotinidase activity, have been introduced in many countries and have made possible early diagnosis and treatment of many children with metabolic disorders [1–10]. This is widely presumed to lead to improvement in their prognoses, but data are few, and it may not hold true for all disorders [11–13]. There are no randomized controlled studies to support clinical effectiveness of expanded newborn screening, both because of the rarity of the disorders and because of a general belief among experts that newborn screening is beneficial [3].

There is no consensus on which disorders to include in screening panels, which therefore differ among countries; most include various inborn errors in the metabolism of amino acids, fatty acids, and organic acids [13]. New metabolites, tests, and diseases are being added to screening platforms, such as galactose-1-phosphate for GALT [14,15] and assays for lysosomal storage disorders [16]. Although considerable experience has been gained, published results come from relatively few centers. Health technology assessments have been done in a few countries, including the Netherlands [17], the UK [18], Spain [19], and most extensively in the US, where a comprehensive report has evaluated potential disorders to be included in neonatal screening panels according to disease characteristics, screening test performance, and economic considerations [20]. Reports arrive at different conclusions, mostly because of the limited knowledge of clinical effectiveness of expanded newborn screening and different emphasis on the mere availability of a multiplex screening method for introducing screening for a given disorder. More recently, the EU has proposed a European frame for design and execution of neonatal screening, which may cover most legislative, technological and outcome measures [21].

Frequencies of non-PKU metabolic disorders found by expanded newborn screening have varied, but in general the frequencies have been higher than those observed before screening [3,22]. This is true for disorders with a well known severe natural history as well as for disorders where the natural history in a substantial fraction of patients is unresolved or possibly benign, like 3-MCCD and SCADD. The most prevalent non-PKU IEM in Western countries is MCADD [3,4], in which increased frequencies of mutations with both severe and possibly benign phenotypic consequences have been found following the implementation of neonatal screening [23,24].

The false positive rate (FPR) of expanded newborn screening is low for some disorders, like MCADD, but the aggregate FPR for all disorders in a screening panel may not be trivial, and FPR of 0.02–0.38%, or 20–380 newborns in centers screening 100,000 newborns per year, have been reported [1–10]. Parental anxiety caused by false positive results may have been overestimated [25,26], but a low FPR should still be in the front when choosing a disorder to screen for, especially if the disorder in question is benign in many patients.

We now present clinical, biochemical, and statistical results of expanded screening of 504,049 newborns during nine years in Denmark, the Faroe Islands, and Greenland as well as diagnoses and clinical findings in 82,930 unscreened children born in the same period. Frequencies of diagnoses made within the panel of disorders screened for in the decade preceding expanded newborn screening are given also. The expanded newborn screening was performed as a pilot project including a relatively large group of disorders during the first seven years, and the experience obtained during this period was used for the development and implementation of a routine newborn screening program introduced in 2009, which is also described here.

2. Subjects and methods

2.1. Subjects

Until 2002 only PKU, CH, and CT were screened for among newborns in Denmark, the Faroe Islands, and Greenland in an informed dissent set-up. On February 1st, 2002, an expanded newborn screening trial for a panel of disorders detectable by MS/MS profiles of amino acids, acylcarnitines, and hexosemonophosphates was initiated in Denmark, the Faroe Islands and Greenland. With few changes (see below, Tables 1 and 2, and Fig. 1), this trial continued until February 2nd, 2009, when several disorders from the expanded newborn screening trial became part of a routine neonatal screening program (Table 3). During the trial period (2002–2009), newborns were recruited, after informed parental consent, to additional expanded newborn MS/MS screening in conjunction with the routine screening for PKU, CH, and CT. Families were informed about the project in a written information package about neonatal screening as well as by the person, who did the sampling (mostly midwives, nurses or laboratory technicians). This person also obtained consent, which was documented on the filter paper blood spot (see also www.ssi.dk/nyfoedte). Filter paper blood samples were only processed if the attached form was clearly marked with an X in the box "Yes" to expanded newborn screening. Our report covers results obtained from February 1st, 2002, until March 31st, 2011 (pilot project period and routine screening from 2009), and it includes findings from the expanded newborn screening using MS/MS as well as results from screening for BIOTD (after February 2nd, 2009), but not from the screening for PKU, CH, CT, and CAH (the latter added after 2009). The study was approved by the Ethics Committee of Copenhagen and Frederiksberg (KF 01-152/98).

According to Statistics Denmark, which collects population data for all of Denmark, the Faroe Islands, and Greenland, 586,979 children were born in Denmark, the Faroe Islands, and Greenland from February 1st, 2002, until March 31st, 2011 (http://www.statistikbanken.dk/statbank5a/default.asp?w=1120). Close to 100% of the children took part of the routine screening program offered during that period. The number of children participating in the expanded newborn screening program was 504,049; accordingly, 82,930 children did not participate (Fig. 1). The percentage of newborns participating in the expanded screening trial varied from 65% in the first years to 85% in the last years of pilot screening. Most children not participating in expanded screening were born during the first years of pilot screening; after February 2nd, 2009, when routine expanded screening was introduced, close to 100% of newborns were screened. Failure to inform parents about the pilot study was the major reason for the low initial coverage, and the fraction actively declining expanded