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The First Results of Extended Newborn Screening in the Majority and the Roma Ethnic Group in Slovakia

Svetozár Dluholucký [1,2] *, Mária Knapková [1]

[1] Newborn Screening Centre of Slovak Republic, Children Faculty Hospital, 97409 Banská Bystrica, Slovak Republic

[2] Faculty of Health Slovak Medical University, in Bratislava, Sládkovičova, 21, 97405 Banská Bystrica, Slovak Republic

Address correspondence to: svetozar.dluholucky@dfnbb.sk; phone: +421905449038, +421484726566

Abstract:
Authors present The First Results of the National Extended Newborn Screening (ENS) in Slovakia in the majority (M) and the Roma (R) ethnic populations. The follow-up of the ethnicity has been introduced in Newborn Screening for Cystic Fibrosis (NSCF) and after to entire ENS program comprising of 23 Hereditary Metabolic Disorders (HMD). Results: In 2013-2015, a total of 165,648 newborns were investigated in ENS, 23,321 of them (15,3%) were the R ethnic group, a total of 313 positive cases were discovered (total ENS prevalence = 1:529, M=1:758, R=1:198). In the R ethnic group, there was slightly higher prevalence in cong. hypothyreosis (CH), only one case of CF, and no cases of CAH in the R ethnic group. The ENS prevalence of HMD detected by MS/MS was expressively higher in the R ethnic group than in M group (M=1:1670 vs. R=1:234, OR:7,13). Significant differences in the prevalence of individual types of HMD were found. Whereas the PKU and spectrum of aminoaciduria and organic acidurias dominate in the M group, the fatty acids oxidation disorders (MCAD, SCAD) and carnitine defects (CUD) were frequent in the R newborn group.
Conclusion: Despite the presented results are preliminary, the ethnic approach to ENS is enabling the recording of the ethnic differences in the screening prevalence of individual disorders, which would be missing during unitary approach.

KeyWords: extended newborn screening; ethnic screening differences; Roma ethnicity

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Introduction

Newborn screening (NS) in Slovakia was started in 1985. The first screening was the detection of congenital hypothyreosis (CH 1985), followed by phenylketonuria (PKU 1995) and congenital adrenal hyperplasia (CAH 2003) /1/. During a pilot study, before the screening was extended by cystic fibrosis (NSCF – newborn screening for cystic fibrosis), an elevated level of the immunoreactive trypsinogen (IRT) screening test was found in as many as 32% of the newborn babies in the roma ethnic (R) group, compared to the majority (M) population. This necessitated the setting-up of different cut-off limits for the majority and the roma population and hence the monitoring of ethnicity of the newborn population. The NSCF was launched nationwide in 2009 and these results were published previously /2,3/. From 1st January 2013, the newborn screening was extended by the introduction of a tandem mass spectrometry (MS/MS) of 20 inborn errors of metabolism (IEM). The monitoring of the ethnicity of the population, initially for the needs of NSCF, has also allowed us to evaluate of the total and ethnic incidences of other IEM. The aim of this work is to present the first results of the NS from 2013 to 2015.

Material and Methods

The Extended Newborn Screening (ENS) was carried out in a Neonatal Screening Centre at the Children Faculty Hospital Banská Bystrica, Slovakia. From 2013, the ENS included 20 types of IEM, as shown in **Table 1**, using the MS/MS method, 10 of them as an official national NS confirmed by Ministry of Health of Slovak Republic and another 10 as a pilot prospective study. Together with CH, CAH and CF, this represents a total of 23 disorders. The paper presents the results obtained from 1 January 2013 to 31 December 2015. All the cases presented, detected in the ENS have been diagnostically verified and definitively confirmed by the specialized regional centres for metabolic diseases, including genetic testing.

Table 1. Spectrum of IEM in extended newborn MS/MS screening since 1. January 2013

IEM reg	**abb.**	**IEM pil. Study**	**abb.**
phenylketonuria/ hyperphen.	PKU/HPA	thyrosinemia type I and II	Tyr I, II
leucinosis maple sir urin dis	MSUD	Hypermethyoninemia	Met
isovaleric acidemia	IVA	propionic acidemia	PA
glutaric acidemia type I	GAI	methylmalonic acidemia	MMA
medium chain acylCoA DH	MCAD	Citrulinemia	Cit
very long chain acyl Coa DH	VLCAD	Argininemia	Arg
long chain acyl CoA DH	LCHAD	short chain acyl CoA DH	SCAD
carnitine plam. tranferase 1	CPT 1	3-methyl crotonyl CoA deficiency	3MCC
carnitine plam. transferase 2	CPT 2	hydroxymethylglutaril CoA 1 deficiency	HMG
carnitine - acylcarnitine transl.	CACT	carnitine uptake defect	CUD

Results

The ENS results are comprehensively presented in **Table 2**.

Table 2. Results of extended newborn screening in Slovakia 2013 - 2015

Data	Total pop.	Major	Roma	OR
Absol. n.	**165648**	**140327**	**25321**	
M/R%	100	84,70%	14,30%	
IEMabs.	**192**	**84**	**108**	
incid.	862	1670	234	
prev.10^4	11,59	5,98	42,65	OR:7,13
CH	**87**	**68**	**19**	
incid.	1904	2063	1333	
prev.10^4	5,25	4,84	6,56	OR:1,35
CAH	**10**	**10**	**0**	
incid.	16565	16565	<25321	
prev.10^4	0,6	0,71	0	
CF	**24**	**23**	**1**	
incid.	6902	6101	25321	
prev.10^4	1,45	1,64	0,39	
total abs	**313**	**185**	**128**	
tot.incid.	**529**	**758**	**197**	
prev.10^4	**18,9**	**13,2**	**50,5**	**OR:3,83**

Abb.: OR – Odd ratio

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During the three years of screening (2013-2015), a total of 165,648 newborns were examined which, according to the results of the National Statistical Register of births, is virtually 100% of the population of live births. Of these, 25,321 (15.4%) were roma (R) children and 140,327 (84.7%) children of the major (M) ethnicity. In the group of IEM, there were 192 cases detected in the whole spectrum of disorders, e.g. the overall IEM incidence is 1: 863. Of these, 84 cases were in M group (1 : 1,671) and even 108 cases in R group (1 : 234). Eighty seven cases of CH (incidence 1 : 1,904) was detected, 68 cases were in M group
(1 : 2,063) and 19 cases in R group (1 : 1,333) - odd ratio: 1.35. Ten 10 cases of CAH were detected (1 : 16,565), all of them in the M group. No case of CAH was detected in the R group. Similarly, from 24 detected cases of CF (1 : 6,902), 23 cases were in M group
(1 : 6,101), and only one case was in R group. Overall, the ENS detected and confirmed the 313 positive cases, which the screening incidence in total population is 1: 529. After ethnic groups the overall incidence in M group is 1 : 758, and in R group is 1 : 197. Difference between M and R groups are even more obvious when these data are presented as prevalence (n/10^4 **Table 3,).**

Table 3. Total and ethnic prevalence of screening disorders (n to 10^4)

prev.10^4	**Major**	**Roma**	**Total**
HMD	**5,98**	**42,65**	**11,59**
CH	**4,84**	**6,56**	**5,25**
CAH	**0,71**	**0**	**0,6**
CF	**1,64**	**0,39**	**1,45**
Total	**13,2**	**50,5**	**18,9**

IEM prevalence as well as the total screening prevalence in group R is nearly fourfold compared to group M, whereas the prevalence of CF and CAH is low in the Roma population. Differences in IEM category between groups M and R are even more significant. The most common disorders of the whole set **(Table 4)** are SCAD, PKU, CUD and MCAD.

Table 4. Absolute numbers of IEM detected by newborn screening after ethnicity

Disorder	Major	Roma	Total
PKU	32	8	40
MCAD	6	9	15
GA1	2	0	2
LCHAD	1	0	1
IVA	1	0	1
MSUD	1	0	1
Met	1	0	1
Tyr	4	2	6
3MCCD	8	1	9
MMA	2	1	3
CUD	20	16	36
Cit	2	0	2
SCAD	2	69	71
PA	0	2	2
NKH*	1	0	1
Gal	1	0	1
Total	84	108	192

* Nonketotic hypeglycinemia

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When the total group is divided after ethnicity, the distribution of IEM is even more obvious. PKU is the most common disorder in the majority (M) ethnic group, followed by CUD and there is a wide range of IEMs (**Figure 1**). The most common disorder in the R ethnic group is SCAD, followed by CUD, MCAD, and PKU is in fourth place **(Figure 2)**. In this ethnic group, the spectrum of IEMs is not so large as in M ethnic group.

Figure 1. Spectrum of IEM in the majority newborns

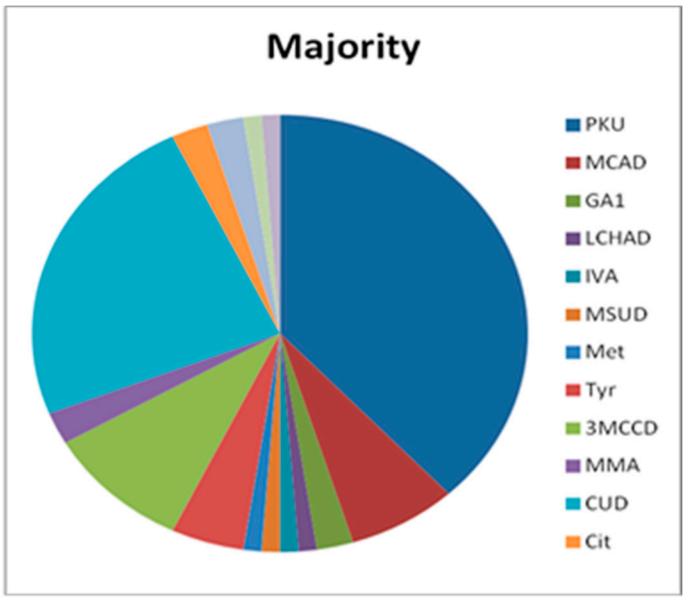
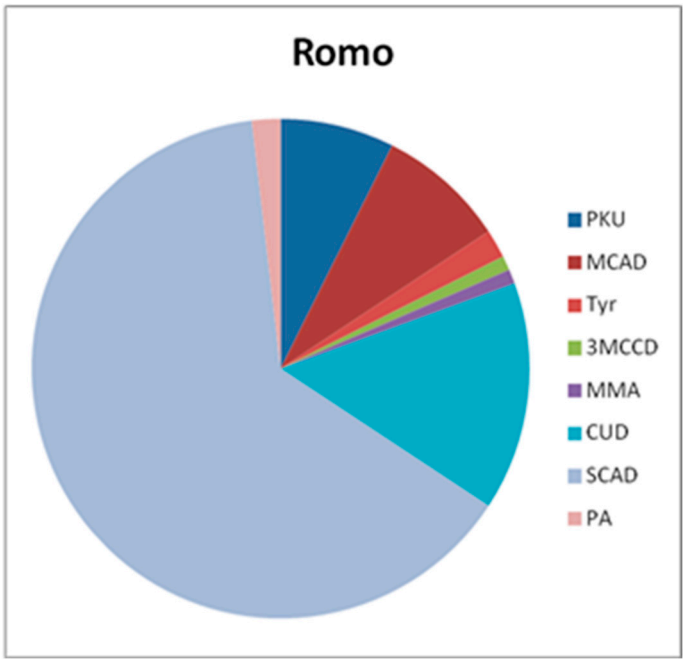


Figure 2. Spectrum of IEM in the Roma newborns



These differences become more obvious when they ar converted to prevalence (**Table 5**).

Table 5. Prevalence (n to 10^4) of the most frequent IEM in total newborn population and after the ethnicity

HMD	Majority	Roma	Total
PKU	2,28	3,16	2,41
MCAD	0,42	3,35	0,9
CUD	1,43	6,32	2,17
SCAD	0,14	27,25	4,29
Total	5,99	42,65	11,59

Discussion

The introduction and monitoring the ethnicity of newborns was required because the NSCF showed significantly higher IRT value and thus the need of different cut-off limit in roma newborns (2,3). It was introduced after the approval of the ethics committees of the Children Faculty Hospital Banská Bystrica and the Slovak Ministry of Health. The second moment in expanding the range of IEM in the ENS was the fact that, in addition to the officially planned 10 IEMs, the used MS/MS software enabled us to direct detection of 10 other IEMs during the regular testing of the dry blood sample. This entire spectrum of IEMs complies with Wilson-Jungner eligibility criteria for NS, and many of them were included in the regular NS of many European countries /4,5/ and in US NS /6/. The implementation of follow-up the ethnicity during ENS brought us many new and unexpected results. Before 2013, results of NS in Slovakia were evaluated for the whole newborn population. However at that time, the high incidence of CH in roma population in the eastern region of Slovakia was reported /7/. Anecdotal claims about the low incidence of CF in the roma population in Slovakia did not occur, until the introduction of NSCF, as a subject of serious study. At the same time, in the world elevated IRT value has been noted in African American newborns /8,9/, and there were found differences in CF incidence in these ethnic groups /6/. Despite, the relation of significantly higher incidence of sudden infant death (SID /10/), which may be related to some IEMs /11,12/, has not been analysed yet. Recent studies of the roma population in Central Europe also point to differences in the roma population in Slovakia in comparison to roma populations of neighbouring countries /13,14,15/. The Slovak Republic (SK), with its location in the centre of Europe and five million inhabitants, has a relatively homogeneous population of predominantly Caucasian ethnicity. The most significant autochthonous minority is the roma ethnic group, representing about 10% of the population (absolutely cca 500,000 people). Any differences in certain demographic data (e.g. infant mortality rate, under 5 mortality rate, etc.) in this community has been attributed to disadvantaged social conditions and a different way of life /16/. Studies, concerning the hereditary origin of these health handicaps are rare. Slovakian roma children showed a significantly high incidence of congenital glaucoma, which is rare even among the roma populations of neighbouring countries /14,17/. The ENS introduced in Slovakia within the first three years revealed marked differences in the occurrence of various disorders in the majority and the roma ethnic group. Although these results are preliminary, they are roughly comparable with the results of the California study of the ethnic / racial differences in the prevalence of IEMs. Low, or zero incidence of CF and CAH, and contrasting high incidence of MCAD, SCAD and CUD in the Slovak roma population are both similar to California's findings in newborns from the Middle East and India /6/. Namely these regions are the provenance of the European roma population /14,15/. The second ENS issue discussed, is the spectrum of IEMs included in the screening, and the effort of its unification /4,5,18,19/. Even in EU countries the range of screened disorders varies from 4 to 30 IEMs /5/. Especially NS of CUD and SCAD is a matter of debate. Both these disorders have the large variability in the incidence, heredity, clinical expression, symptoms, and the need of treatment /20,21,22/. The extraordinary high screening incidence of these disorders has been found in our roma group of newborns. This opens up a number of issues, notably: 1./ What is the range of

symptomatic and asymptomatic forms of identified cases of SCAD, are there differences in their genetic background between the majority and the roma ethnic group? 2./ Will the long-term ENS results confirm the differences found? 3./ Will it be possible to differentiate the forms– especially SCAD and CUD in terms of the need for continuous monitoring and therapeutic intervention? All of these questions will be solved in the following studies. However, now it is obvious that the high prevalence of the CUD and SCAD is the sufficient reason to include them into regular ENS. Confirmation of accuracy of these steps can be expected from the further results.

Conclusion

The ethnically evaluated ENS in Slovakia, after a short period of time, found significant differences in the incidence and prevalence of almost all disorders, especially in the IEM group, between the majority and roma population of newborns, which did not show up within the global evaluation study. The significance of the differences observed is that they allow to focus purposefully on the curative and preventive care, especially for children living in socially disadvantaged areas. It also creates opportunities for further research into the etiopathogenesis of these differences.

Author´s Contributions Svetozár Dluholucký conceptualized and designed the study, continuously followed the study and corrected the individual deviations in its course, drafted and finalised the manuscript and approved its for submission. Mária Knapková supervised the screening study, designed the data collection, supervised the data collection, critically reviewed the manuscript, and approved the final manuscript to be submitted .

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