NEWBORN SCREENING IN NORTH OSSETIA IN 2023–2024

Zinchenko RA[1]✉, Tebieva IS[2,3], Zakharova EYu[1], Shchagina OA[1], Lotnik EE[1], Gabisova YuV[3], Khokhova AV[2,3], Bakin NV[1], Marakhonov AV[1]

[1] Research Centre for Medical Genetics, Moscow, Russia
[2] North-Ossetian State Medical Academy, Vladikavkaz, Russia
[3] Republican Children's Clinical Hospital, Vladikavkaz, Russia

Mass screening of newborns for 36 hereditary diseases in the Russian Federation will enable the reduction of childhood disability and mortality from hereditary disorders, as well as the identification of all-Russian and regional population-genetic features of the screened disorders. The study aimed to assess the results of newborn screening (NBS), including expanded newborn screening (ENBS), in the Republic of North Ossetia-Alania obtained between January 1, 2023, and December 31, 2024, as well as to study clinical and population-genetic characteristics of the diseases screened in the region. In phase I of assessment, biochemical testing, tandem mass spectrometry, and DNA diagnostics were performed, and the TREC/KREC levels were determined in 14,994 newborns. In 355 cases (2.36%), positive values were revealed. In phase II, the necessary laboratory and subsequent confirmatory DNA diagnostics were carried out in 324 cases (91.2%): repeated analysis by MS/MS and DNA diagnostics (for hereditary metabolic diseases), immunophenotyping (for primary immunodeficiency states). During the 2-year study, a total of 37 diagnoses were established, which accounted for 0.25% of all children screened in phase I and clearly indicated the program's success and effectiveness. We managed to verify the specific spectrum of mutations characteristic of phenylketonuria (PKU) and medium-chain fatty acid acyl-CoA dehydrogenase deficiency (MCADD). The frequency of the disorder assessed within the framework of newborn screening was determined. The frequency of all PKU forms was 1 : 1153 newborns, and the frequency of MCADD was 1 : 789 newborns surveyed. All children are listed as sick in the medical genetic consultation of the Republic of North Ossetia-Alania; they receive treatment in accordance with the clinical guidelines.

Keywords: newborn screening, expanded newborn screening, hereditary pathology, phenylketonuria, medium-chain fatty acid acyl-CoA dehydrogenase deficiency

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Compliance with ethical standards: the study was approved by the Ethics Committee of the Research Centre for Medical Genetics (protocol No. 7 dated 20 December 2017), it was compliant with the standards of Good Clinical Practice and evidence-based medicine. All patients submitted informed consent to participate in the study.

✉ **Correspondence should be addressed:** Rena A. Zinchenko
Moskvorechie, 1, 115522, Moscow, Russia; renazinchenko@mail.ru

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НЕОНАТАЛЬНЫЙ СКРИНИНГ В СЕВЕРНОЙ ОСЕТИИ ЗА 2023–2024 ГГ.

Р. А. Зинченко[1]✉, И. С. Тебиева[2,3], Е. Ю. Захарова[1], О. А. Щагина[1], Е. Е. Лотник[1], Ю. В. Габисова[3], А. В. Хохова[2,3], Н. В. Бакин[1], А. В. Марахонов[1]

[1] Медико-генетический научный центр имени Н. П. Бочкова, Москва, Россия
[2] Северо-Осетинская государственная медицинская академия Министерства здравоохранения России, Владикавказ, Россия
[3] Республиканская детская клиническая больница, Владикавказ, Россия

Массовое обследование новорожденных в Российской Федерации на 36 наследственных заболеваний позволит снизить детскую инвалидность и смертность от наследственной патологии и выявить общероссийские и региональные популяционно-генетические особенности скринируемой патологии. Целью исследования было оценить результаты неонатального скрининга (НС), включая расширенный неонатальный скрининг (РНС) в Республике Северная Осетия-Алания (РСО-Алания) в период с 01.01.2023 по 31.12.2024, и изучить клинические и популяционно-генетические особенности скринируемых заболеваний в регионе. На I этапе обследования у 14 994 новорожденных проведены биохимические исследования, тандемная масс-спектрометрия, ДНК-диагностика и определение уровня TREK/KREK. В 355 случаях (2,36%) выявлены позитивные значения. На II этапе в 324 (91,2%) случаях проведена необходимая лабораторная и последующая подтверждающая ДНК-диагностика: повторный анализ в МС/МС и ДНК-диагностика (для наследственных болезней обмена веществ), иммунофенотипирование (для первичных иммунодефицитных состояний). В ходе двухлетнего исследования поставлено 37 диагнозов, что составляет 0,25% от всех детей, охваченных скринингом на I этапе, и однозначно свидетельствует об успешности и результативности данной программы. Удалось верифицировать специфический спектр мутаций, характерных для фенилкетонурии (ФКУ) и недостаточности ацил-КоА-дегидрогеназы жирных кислот со средней длиной углеродной цепи (MCADD). Определена частота встречаемости патологии, исследуемой в рамках неонатального скрининга. Частота всех форм ФКУ составила 1 : 1153 новорожденных, а частота MCADD — 1 : 789 обследованных новорожденных. Все дети состоят на диспансерном учете в медико-генетической консультации РСО-Алания, получают лечение в соответствии с клиническими рекомендациями.

Ключевые слова: неонатальный скрининг, расширенный неонатальный скрининг, наследственная патология, фенилкетонурия, недостаточность ацил-КоА-дегидрогеназы, жирные кислоты со средней длиной углеродной цепи

Финансирование: исследование выполнено из средств федерального и регионального бюджетов в части реализации расширенного неонатального скрининга, а также при финансовой поддержке Государственного задания ФГБНУ «МГНЦ» Минобрнауки России и Минздрава РСО-Алания.

Вклад авторов: И. С. Тебиева, Ю. В. Габисова, А. В. Хохова — сбор данных, постановка диагноза; Р. А. Зинченко, И.С. Тебиева — планирование исследования, написание текста; Е. Ю. Захарова, О. А. Щагина, Е. Е. Лотник, Н. В. Бакин, А. В. Марахонов — проведение молекулярно-генетических исследований; Р. А. Зинченко, И. С. Тебиева, Е. Ю Захарова — редактирование текста.

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✉ **Для корреспонденции:** Рена Абульфазовна Зинченко
ул. Москворечье, д. 1, 115522, г. Москва, Россия; renazinchenko@mail.ru

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Newborn screening has been demonstrating its effectiveness in detecting congenital and hereditary disorders (CHDs) at the early preclinical stage for decades. Mass screening of newborns was launched on January 1, 2023, in the Russian Federation (RF). Today, it is divided into newborn screening (NBS) and expanded newborn screening (ENBS). In the regions, NBS is conducted in order to detect five CHDs: phenylketonuria, congenital hypothyroidism, cystic fibrosis, galactosemia, and congenital adrenal cortex dysfunction (PKU, CH, CF, GAL, CACD, respectively). ENBS is implemented in 10 interregional 3A-level ENBS centers in order to detect 29 hereditary metabolic diseases (HMDs), spinal muscular atrophy (SMA), and severe T-cell and B-cell primary immunodeficiency (PID) [1].

The Decree of the Government of the Republic of North Ossetia–Alania (RNO-Alania) approved the regional program of NBS and ENBS implementation; regulations governing this trial were developed; officials responsible for the implementation of all screening phases were determined, and routing of patients within the framework of the existing infrastructure was arranged.

The study aimed to assess the results of NBS and ENBS in the RNO-Alania reported between January 1, 2023 and December 31, 2024, as well as to determine clinical and population genetic characteristics of the diseases screened.

METHODS

In phase I of screening, capillary blood sampling from the newborn's heel was performed on day 2 in full-term babies and on day 7 in preterm babies using two assay sheets: one with five blood spots for NBS and another with three blood spots for ENBS. The referral with supporting data is generated using the following module: Obstetrics and Neonatology Vertical Integrated Medical and Information System (VIMIS AKiNEO). The data on the children at risk are conveyed to the officials responsible for ENBS arrangement in the constituent entity within 24 hours. In the following 48 h, biomaterial re-collection from children at risk is arranged, and the biomaterial is transferred to the 3B level reference center, the Research Centre for Medical Genetics, where the exact diagnosis of CHD is established or ruled out, for confirmatory diagnostics [2].

Initial examination of the children born in the RNO-Alania being part of the NBS, is conducted at the Medical Genetic Consultation (MGC) of the Republican Children's Clinical Hospital of the RNO-Alania. The levels of biochemical markers in whole blood samples are determined by the time-resolved immunofluorescence method using the DELFIA Neonatal (Finland) and FAVR (Russia) reagents in the Victor-2 system (Wallak, Finland). The measurement results are entered into the computer program for data processing and acquisition. To establish the diagnosis, the values of biochemical markers should be as follows: phenylalanine (PA) > 2 mg% — for the diagnosis of PKU; thyroid-stimulating hormone (TSH) > 20 μIU/L — for CH, immunoreactive trypsin (IRT) > 70 ng/mL — for CF, 17-hydroxyprogesterone (17-OHP) > 30 nmol/L — for CACD in full-term babies and > 60 mmol/L — for CACD in preterm babies, galactose > 400 nmol/L (7 μmol/L) — for GAL. Initial examination being part of ENBS is conducted at the Research Institute — Regional Clinical Hospital No. 1 named after Professor S. V. Ochapovsky of the Ministry of Health of the Krasnodar Krai: testing for HMDs (including PKU) is performed by tandem mass spectrometry (MS/MS); testing for SMA and PID is accomplished via qualitative identification of the homozygous deletion of exon 7 in the SMN1 gene and quantification of the TREC, KREC DNA involving the use of the Neoscreen SMA/TREC/KREC system (DNA-Technology TS, Russia).

In phase II of the assessment, the following essential biomaterial samples must be made available for the Research Centre for Medical Genetics:

HMDs — dried blood spots on the assay sheet, liquid blood in the test tube with EDTA (non-frozen, at least 2.5 mL) and urine (at least 5 mL);

SMA — dried blood spots on the assay sheet, liquid blood in the test tube with EDTA (non-frozen, at least 2.5 mL);

PID in phase I — dried blood spots on the assay sheet, in phase II — liquid blood in the test tube with EDTA (non-frozen, two test tubes) for immunophenotyping and DNA diagnostics.

Confirmatory diagnostics is accomplished via repeated analysis of amino acids and acylcarnitines by MS/MS, determination of urinary levels of organic acids by gas chromatography–mass spectrometry (GC–MS), and DNA diagnostics. In the second phase of testing for PID, immunophenotyping (IPT) is performed at the Dmitry Rogachev National Medical Research Center of Pediatric Hematology, Oncology and Immunology of the Ministry of Health of the RF.

RESULTS

A total of 15,153 babies were born in the Republic during the studied period. A total of 15,059 (taking into account those who died before sampling) were to be assessed. Parents of 65 newborns submitted the informed refusal of screening, and blood was collected from 14,994 babies. The survey covered 99.56% of newborns.

In all 14,994 cases, tandem mass spectrometry was conducted in phase I, of those, in 355 cases (2.36%), the screening results were positive.

Since the Republic represents the region with high ethno-cultural diversity and close ties with the Caucasian and Transcaucasian territories, a large number of children are traditionally born there, who later move to other regions of the RF (Chechen Republic, Republic of Ingushetia, Stavropol Krai, etc.) and abroad (South Ossetia, Georgia, Armenia, etc.). In this regard, in 31 cases (8.8%), information about the results of assessment performed within the framework of ENBS was shared with the officials responsible for the implementation of screening in adjacent constituent entities of the RF. Considering this fact, 324 newborns out of 355 were assessed in phase II, which accounted for 91.2%.

Testing for aminoacidopathies

Given the fact that testing for PKU was performed by the biochemical method within the framework of NBS and by MS/MS within the framework of ENBS, we had an opportunity to compare the data of the testing conducted and draw a conclusion about the MS/MS higher informativity in terms of PKU detection.

Based on the DNA diagnostics data, alterations of the *PAH* gene nucleotide sequence typical for PKU in the homozygous and compound heterozygous state were found in 13 patients. The PKU frequency determined based on the tandem mass spectrometry results was 1 : 1165 newborns and 1 : 1153 surveyed newborns (95% CI: 1 : 675 – 1 : 2166). All children are on the MGC dietitian's D list. The dynamic changes in PA levels and the treatment tactics are determined in accordance with the Federal Clinical Guidelines. Only two children need foods for special medical purposes (FSMP), while in other patients, PA levels do not exceed 6 mg% (360 μM/L) [3].

Table 1. Biochemical and molecular genetic characteristics of patients with PKU

№	PA level in phase I mg%/μM/L)*	Phe/Tyr**	FSMP	Ethnicity	Allele 1 (RefSeq NM_000277.3)	Allele 2 (RefSeq NM_000277.3)
1	1.77/142	2.35	–	Ossetians	c.529G>A (p.Val177Met)	c.119C>T (p.Ser40Leu)
2	0.28/131	2.1	–	Ossetians	c.842C>T (p.Pro281Leu)	c.529G>A (p.Val177Met)
3	3.74/273	2.48	–	Ossetians	c.631C>A (p.Pro211Thr)	c.631C>A (p.Pro211Thr)
4	1.98/149	1.78	–	Ossetians	c.529G>A (p.Val177Met)	c.842C>T (p.Pro281Leu)
5	6.77/405	9.05	+	Ossetians	c.1222C>T (p.Arg408Trp)	c.1222C>T (p.Arg408Trp)
6	2.3/145	2.31	–	Ossetians	c.529G>A (p.Val177Met)	c.1222C>T (p.Arg408Trp)
7	4.79/321	3.66	–	Ossetians	c.842C>T (p.Pro281Leu)	c.722G>A (p.Arg241His)
8	2.4/140	2.2	–	Armenians	c.1208C>T (p.Ala403Val)	c.506G>A (p.Arg169His)
9	38/550	8.8	+	Ossetians	c.842C>T (p.Pro281Leu)	c.1222C>T (p.Arg408Trp)
10	1.9/143	1.98	–	Ossetians	c.533A>T (p.Glu178Gly)	c.490A>G (p.Ile164Val)
11	2.73/286	4.46	–	Female Georgian/Ossetian	c.842C>T (p.Pro281Leu)	c.631C>A (p.Pro211Thr)
12	***/240	2.9	–	Ossetians	c.898G>T (p.Ala300Ser)	c.631C>A (p.Pro211Thr)
13	***/141	2.15	–	Ossetians	c.842C>T (p.Pro281Leu)	c.529G>A (p.Val177Met)

Note: * — PA reference range — 0–2 mg% for NBS, 0–120 μM/L for ENBS; ** — Phe/Tyr ratio reference range 0.25–6.5; *** — not tested for PKU in the MDC of the Republican Children's Clinical Hospital of RNO-Alania due to the lack of reagents.

Biochemical and molecular genetic characteristics of the patients identified are provided in Table 1.

The most common was the "severe" mutation c.842C>T (p.Pro281Leu), in which the residual activity of the PAH enzyme is 2%, the number of heterozygous alleles is 6, homozygous — 0. Among the general sample, the frequency of occurrence is 23%, among representatives of the Ossetian nationality — 27%.

The second most frequent genetic variant was c.529G>A (p.Val177Met), data on PAH activity are not presented in the literature, the frequency of heterozygous carriage is 5, which amounted to 19% in the general sample and 22% among the titular nation. In third place were two variants: "severe" mutation c.1222C>T, (p.Arg408Trp) (residual PAH activity — 2%) and "mild" c.631C>A (p.Pro211Thr) (residual PAH activity — 72%): 1 homozygous case and 2 heterozygous carriers. They accounted for 15% of the total sample and 18% among Ossetians. The remaining variants are found in single heterozygous variants and demonstrate high residual activity of the PAH protein. Patients do not require diet therapy, and therefore the mutations can be classified as "mild" [4, 5]. Thus, out of 13 patients, only two (15.3%) with genotypes with two severe mutations (R408W/R408W and P281L/R408W) require diet therapy.

Impaired mitochondrial fatty acid β-oxidation

The diagnosis belonging to the group of hereditary mitochondrial fatty acid β-oxidation disorders (FAODs) was established in 19 patients based on the MS/MS data and DNA diagnostics: medium-chain fatty acid acyl-CoA dehydrogenase deficiency (MCADD).

The frequency of this disorder in the RNO-Alania was 1 : 789 (95% CI: 1 : 506 – 1 : 1310). The MS/MS data and molecular genetic characteristics of patients with MCADD are provided in Table 2.

In 20 newborns, acylcarnitine levels significantly exceeding reference values were revealed in phase II of diagnostics. However, in case 18, one heterozygous c.985A>G (p.Lys329Glu) mutation was found. The whole genome sequencing in the "trio" format is scheduled for this family, and the case is not considered in the further discussion.

Among 19 patients with two verified mutations, 16 are Ossetians, who were not born into consanguineous marriages. Patients 15 and 16 were monozygotic twins, their parents were Russians. Patient 12 was born into an interethnic marriage between the Ossetian and the female Tabasaran.

In five cases, the c.388-19T>A variant was in the homozygous state. Furthermore, a total of 11 compound heterozygous carriers were identified, which accounted for 55.26% of the entire sample and about 65.62% of the titular nation representatives.

The c.985A>G (p.Lys329Glu) variant ranking second in frequency was found (in the homozygous state in one patient and in the heterozygous carrier state in four cases), which accounted for 15.78% in the entire sample and 18.75% in Ossetians.

The c.134A>C (p.Gln45Arg) ranking third in frequency was found in the compound heterozygous state in four cases, accounting for 10.52 and 12.5% in the entire and titular groups, respectively.

Other variants are sporadic; these account for 18.44% in the entire sample and 3.13% in Ossetians.

It should be noted that the most pronounced changes in the C6, C8, and C10 levels (between 9 and 22 μmol/L) were observed in those having genotypes containing the c.985A>G (p.Lys329Glu) and c.388-19T>A variants in both homozygous and compound heterozygous state.

All children are in the MGC dietitian's and geneticist's D list. The dynamic changes in acylcarnitine levels are determined by MS/MS in accordance with the generally accepted guidelines. The children's condition was stable, and no metabolic crises, hypoglycemic conditions were observed. The identified patients' parents underwent Sanger sequencing in the "trio" format within the framework of the supplementary agreement between the Republican Children's Clinical Hospital of the RNO-Alania and the Research Centre for Medical Genetics; the parents are healthy carriers of the variants identified. During medical genetic counseling of families, prevention of long periods of fasting was discussed, especially in children having intercurrent infectious diseases, in order to prevent metabolic crises, as well as the need for immediate hospitalization in the Republican Children's Clinical Hospital and glucose infusion in case of metabolic crisis [4, 6].

One more case of detecting a disease from the organic aciduria group is as follows: the diagnosis of 3-methylcrotonyl-CoA carboxylase deficiency in a Kumyk child having the MCCC2

Table 2. MS/MS data and molecular genetic characteristics of patients with MCADD

№	Biochemistry indicators based on MS/MS data*	Ethnicity	Genotype (RefSeq NM_000016.6)
1	C6 — 1,04; C6DC — 0,34; C8 — 3,80; C10 — 0,52; C10:1 — 0,21	Ossetians	c.388-19T>A /c.388-19T>A
2	C6 — 1,74; C6DC — 0,52; C8 — 9,78; C8:1 — 0,17; C10 — 0,95; C10:1 — 0,49; C10:2 — 0,03	Ossetians	c.134A>C (p.Gln45Arg)/c.388-19T>A
3	C6 — 1,11; C8 — 4,98; C10 — 0,65; C10:1 — 0,30 + (Phe — 172)**	Ossetians	c.388-19T>A/ c.388-19T>A
4	C6 — 1,50; C6DC — 0,51; C8 — 6,46; C10 — 0,93; C10:1 — 0,45	Ossetians	c.388-19T>A/c.999_1011dup(p.Gln338Ter)
5	C6 — 3,53; C6DC — 1,02; C8 — 17,10; C8:1 — 0,23; C10 — 1,50; C10:1 — 0,58	Ossetians	c.985A>G (p.Lys329Glu)/c.985A>G(p.Lys329Glu)
6	C6 — 0,56; C8 — 1,57; C10:1 — 0,25	Ossetians	c.388-19T>A/c.999_1011dup(p.Gln338Ter)
7	C6 — 0,92; C6DC — 0,47; C8 — 5,25; C10 — 0,83; C10:1 — 0,26	Ossetians	c.388-19T>A/c.388-19T>A
8	C6 — 2,38; C6DC — 0,88; C8 — 12,95; C10 — 1,2; C10:1 – 0,36	Ossetians	c.985A>G (p.Lys329Glu)/c.388-19T>A
9	C6 — 1,22; C6DC — 0,41; C8 — 6,70; C10 — 0,82; C10:1 — 0,57	Ossetians	c.388-19T>A/c.985A>G (p.Lys329Glu)
10	C6 — 1,52; C6DC — 0,50; C8 — 6,10; C10 — 0,77; C10:1 — 0,38	Ossetians	c.388-19T>A/c.388-19T>A
11	C6 — 2,13; C6DC — 0,53; C8 — 9,11; C10 — 1,20; C10:1 — 0,57	Ossetians	c.388-19T>A/c.388-19T>A
12	C6 — 1,50; C6DC — 0,60; C8 — 9,79; C10 — 1,19; C10:1 — 0,44	Ossetian/female Tabasaran	c.388-19T>A/c.985A>G (p.Lys329Glu)
13	C8 — 0,45; C10 — 0,48	Ossetians	c.1091T>C, p.(Ile364Thr)/ c.388-19T>A
14	C6 — 1,31; C8 — 3,76; C10 — 0,44; C10:1 — 0,31	Ossetians	c.388-19T>A/c.461T>G, p.Leu154Trp
15	C6 — 1,24; C8 — 3,59; C10 — 0,41; C10:1 — 0,39	Ossetians (monozygotic twins)	c.388-19T>A/c.400_401delATinsCA, p.(Ile134His)
16	C6 — 1,38; C8 — 4,13; C10 — 0,49; C10:1 — 0,45		c.388-19T>A/c.400_401delATinsCA, p.(Ile134His)
17	C6 — 2,15; C8 — 10,5; C10 — 1,18; C10:1 — 0,45	Ossetians	c.134A>C (p.Gln45Arg)/c.388-19T>A
18	C6 — 0,38; C8 — 0,59; C10 — 0,73; C10:1 — 0,25	Ossetians	c.985A>G (p.Lys329Glu)/***
19	C6 — 3,57; C8 — 22,4; C10 — 1,97; C10:1 — 0,72	Ossetians	c.134A>C (p.Gln45Arg)/c.985A>G (p.Lys329Glu)
20	C6 — 1,28; C8 — 5,13; C10 — 0,61; C10:1 — 0,316	Ossetians	c.388-19T>A/c.134A>C (p.Gln45Arg)

Note: * — reference values for the studied indicators (μM/L): C8 — 0–0.26; C10 — 0–0.32; C6DC — 0–0.45; C10:1 — 0–0.14; ** — case of detecting MCADD and PKU; *** — mutation not found when performing the ACADM whole gene sequencing, the whole genome sequencing is recommended.

c.1082G>A (p.Arg361Gln) mutation in the homozygous state. The child's condition is stable, and the development is age-appropriate. The child is on the MGC dietitian's D list in the Republican Children's Clinical Hospital.

Testing for spinal muscular atrophy

Five newborns were included in the group at risk in phase I of ENBS during the studied period, among them the diagnosis of SMA was established in three cases. The SMA frequency was 1 : 4998 newborns (95% CI: 1 : 1711 – 1 : 24,235), which was generally consistent with the data provided in the world's and domestic literature. The average global prevalence of SMA is 1 : 6000 – 1 : 10,000 newborns, the prevalence in Saudi Arabia is 1 : 7000, the prevalence in Egypt and Libya is 1 : 12,500, and the prevalence in the RF is 1 : 5184 [7]. The SMA diagnostics is accomplished in accordance with the existing diagnostic algorithms within the framework of the clinical guidelines [8].

Table 3 provides the SMA patient assessment results and the therapy prescribed.

Based on the assessment results, in accordance with the guidelines of the Federal Council of the National Medical Research Center for Children's Health, two patients were administered Onasemnogene abeparvovec at the age of 1.5 months and 1 month; Risdiplam was recommended in one case.

The patients are on the neurologist's and geneticist's list.

Testing for primary immunodeficiency (PID)

In the studied period, the diagnosis of PID was established in none of the children in the region. However, in phase I of ENBS, 30 cases of low TREC/KREC were reported, among those, seven children died before blood re-sampling later (if premature), before reaching the post-conceptual age of 37 weeks. In 23 cases, the further scheduled assessment showed that all indicators were within reference ranges. It is assumed that the prevalence of PID in the region is below 1 : 14 994 newborns (95% CI: 0 – 1 : 4065).

Testing for five CHDs within the framework of newborn screening

Testing was performed in 14,994 cases. We have earlier reported epidemiological and molecular genetic characteristics of the disorders screened in the RNO-Alania [9]. The values provided that have been acquired during conventional biochemical screening confirm the results obtained previously for CH, CF, GAL, and CACD. Table 4 presents the results of the 2-year study conducted within the framework of NBS at the MGC of the Republican Children's Clinical Hospital of the

Table 3. SMA patient assessment results, timing of the beginning therapy and drugs used for treatment

Year	SMN1 gene copy number	SMN2 gene copy number	AAV9 carrier state	Therapy	Beginning of treatment (months)
2023	0	3	neg*	Onasemnogene abeparvovec	1.5
2023	0	4	neg	Risdiplam	1.5
2024	0	2	neg	Onasemnogene abeparvovec	1

Note: neg* — negative.

Table 4. NBS results, 2023–2024

Year	PKU		CH		CF		GAL		CACD	
	n	F (95% CI)	*n*	F (95% CI)	*n*	F (95% CI)	*n*	F (95% CI)	*n*	F (95% CI)
2023	3	1 : 2999 (1 : 1285 – 1 : 9235)	2	1 : 2999 (1 : 1285 – 1 : 9235)	0	0 (0 – 1 : 4065)	0	0 (0 – 1 : 4065)	1*	1 : 7500 (1 : 2076 – 1 : 61,904)

Note: *n* — number of patients, F — frequency, 95% CI — 95% confidence interval; * — CACD caused by 3β hydroxysteroid dehydrogenase deficiency that is rare in other world's populations and the RF, but typical for Ossetians, has been detected.

RNO-Alania, showing a low rate of PKU detection based on NBS compared to ENBS.

Comparison of NBS and ENBS in the diagnosis of PKU shows higher sensitivity of MS/MS contributing to the identification of patients with mild PKU having confirmed genetic variants in the *PAH* gene, who need monitoring and clinical follow-up.

DISCUSSION

Aminoacidopathies

We had an opportunity to assess PA levels (among 28 other indicators) in the MS/MS format within the framework of ENBS. The data obtained have radically changed the existing knowledge about the PKU population genetic features in the region. Thus, in 2012, PKU frequency in the Republic was interpreted as 1 per 23,000 newborns. This suggested a low prevalence of the disorder in the region, since the diagnosis of PKU was considered as a variant of severe hyperphenylalaninemia (HPA) with the PA levels above 10 mg%, in which mandatory nutritional therapy is prescribed [10]. Later, when cooperating with the Research Centre for Medical Genetics, we had an opportunity to perform DNA diagnostics in all the children identified based on the screening results (PA levels above 2 mg%) and included in the list due to the diagnosis of HPA. The testing results have showed that the frequency of all PKU forms turned out to be 1 : 4864 (95% CI), while the frequency of cases with severe variants requiring nutritional therapy was 1 : 22,374 (95% CI), and that of mild forms – 1 : 6216 (95% CI). The range of molecular genetic features of the disorder also turned out to be specific due to common nature of two mutations, c.842C>T (p.Pro281Leu) (PAH residual activity 0–2%) and c.631C>A (p.Pro211Thr) (PAH residual activity 72%), which are not that frequent anywhere in the world. Furthermore, we have earlier reported the p.Pro281Leu and p.Pro211Thr carrier state in the population with the frequency of 1 : 26 (95% CI), which allowed us to assume considerably higher prevalence of this aminoacidopathy in the RNA-Alania [5, 10], as confirmed by the ENBS results.

The 2-year study conducted within the framework of NBS revealed five PKU cases, and that conducted within the framework of ENBS proved the fact of high prevalence of mild PKU in the region — 1 : 1363 newborns (95% CI), high frequency of all PKU forms — 1 : 1153 (95% CI) and 1 : 7497 (95% CI) for severe forms, for which variants of two severe mutations were reported.

Organic acidurias

According to various sources, the prevalence of MCAD deficiency in the countries of Europe and the USA is 1 : 8300 — 1 : 15,000 newborns. Based on the results of our study, the prevalence of the disorder is 1 : 789 surveyed newborns. According to the literature data, the frequency of this HMD in the world is as follows: 1 : 22,000 in the Czech Republic, 1 : 27,139 in Norway, higher frequency of 1 : 4900 — 1 : 8500 is reported for Germany, 1 : 23,000 in Italy, 1 : 10,000 — 1 : 30,000 in the USA, and the highest frequency of 1 : 4000 is reported for Qatar [11–12]. Exact epidemiological data for Russia have not been published, but preliminary calculations have revealed no important specifics [4].

In 90–95% of cases, a point mutation in exon 11, in which adenine is replaced by guanine at position 985 of the gene (c.985A>G), is found. Other genetic variants that are much less common have also been reported [13–16]. High prevalence of the c.985A>G carrier state has been shown (1 : 52 in Switzerland, 1 : 58 in the UK), along with its decrease from north to south, which is likely to result from the "founder effect" in the ancient German population [17].

The clinical picture of the disease is extremely variable: from asymptomatic to the rapidly developing severe disease [6, 18].

According to the data obtained during ENBS implementation, the unique range of the *ACADM* gene variants was revealed in the region. Thus, the c.985A>G (p.Lys329Glu) variant that is most common according to the data of the world's and domestic literature [4, 15, 19, 20], in our study has been reported for only one case in the homozygous state and for four cases in the compound heterozygous state, i.e. it accounts for only 15.78% in the entire sample and 18.75% in Ossetians. While the c.388-19T>A variant turned out to be the most common in the studied population: 55.26% in the entire sample and about 65.62% in the titular nation representatives. This variant is not found in population databases (gnomAD without frequency). ClinVar contains the mutation description and links to two studies out of the large number of reports in the world's literature, where the disorder is mentioned [16, 21].

The c.388-19T>A was first described in 2012 based on the results of the large-scale Danish study focused on MCADD as previously unregistered and identified based on the screening results of two newborns (unrelated) with the c.388-19T>A/c.244_245dup genotype [21]. It has been proven that mutational sequence alterations occur in the *ACADM* gene intron 5. These dramatically decrease the strength of the wild-type acceptor

splice sites, while generating new competing, stronger splice sites, causing serious cleavage disorders, up to mRNA degradation resulting from the nonsense-mediated decay, and directly altering the encoded ACADM protein amino acid structure.

To date, all the patients identified are under the age of three years, but metabolic crises have not been reported in any of them. This can be associated with mild variants of the mutations identified and the efforts of parents in terms of preventing long fasting periods.

The fact should be noted that no MCADD cases were reported in the Republic before the beginning of the screening period, but patients with hypoglycemic conditions, unclear metabolic crises, especially against the background of respiratory or acute intestinal infectious diseases, were, of course, encountered in the practice of pediatric endocrinologists, pediatricians, infectious disease specialists, and resuscitators. However, the causes of these conditions were not verified due to the lack of the possibility of conducting MS/MS in the region and insufficient physicians' awareness about this disorder.

In our opinion, a very interesting case is a rare combination of PKU and MCADD in one patient (No. 6 in Table 1 and No. 3 in Table 2), who does not need nutritional therapy with limited PA intake and who has no metabolic crises due to the presence of "mild" mutations associated with both disease entities.

CONCLUSIONS

In general, NBS and ENBS implemented in the RNO-Alania can be acknowledged as an effective method for preclinical diagnosis of CHDs, which is considered to be effective when detecting 0.1% of abnormality in the entire cohort of surveyed children. In our study, 37 established diagnoses account for 0.25% of all the children screened in phase I, which clearly demonstrates the program's success and effectiveness. We have shown higher MS/MS sensitivity in detection of mild PKU compared to the standard biochemical method used during NBS. On the one hand, diagnostics in the tandem mass spectrometry format has considerably reduced the percentage of false positive results. On the other hand, the detection rate of this disorder has increased, even against the background of PA values that are slightly above the standard values. During the study, we have also managed to verify the range of mutations typical for PKU and MCADD and determine their frequency in the region. The frequency of all PKU forms is 1 : 1153 newborns, and the MCADD frequency is 1 : 789 newborns. The opportunities that are presented to the scientific community and practitioners as the scientific and technical progress evolves, specifically the ENBS introduction, directly affect the evolution of our views regarding mutations associated with CHDs and their population genetic features in various ethnic groups. Comprehensive assessment of patients and their parents conducted within the framework of ENBS not only contributes to preclinical diagnosis and timely start of treatment of rare disorders, which have remained undiagnosed until now, but also makes it possible to inform the sick child's parents about further family planning and prevention of the CHD spread across the population when providing medical genetic counseling.

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