Oznaka poročila: ARRS-CRP-ZP-2018/42

ZAKLJUČNO POROČILO O REZULTATIH CILJNEGA RAZISKOVALNEGA PROJEKTA

A. PODATKI O RAZISKOVALNEM PROJEKTU

1.Osnovni podatki o raziskovalnem projektu

Šifra projekta	V3-1505
Naslov projekta	Analiza in razvoj področja redkih bolezni v Sloveniji
Vodja projekta	13023 Tadej Battelino
Naziv težišča v okviru CRP	2.1.3 Analiza in razvoj področja redkih bolezni v Sloveniji
Obseg efektivnih ur raziskovalnega dela	821
Cenovna kategorija	C
Obdobje trajanja projekta	10.2015 - 09.2017
Nosilna raziskovalna organizacija	312 Univerzitetni klinični center Ljubljana
Raziskovalne organizacije - soizvajalke	2334 Univerza v Mariboru, Medicinska fakulteta 3135 Splošna bolnišnica Slovenj Gradec 3333 NACIONALNI INŠTITUT ZA JAVNO ZDRAVJE
Raziskovalno področje po šifrantu ARRS	3 MEDICINA 3.05 Reprodukcija človeka
Družbeno-ekonomski cilj	07. Zdravje
Raziskovalno področje po šifrantu FORD/FOS	3 Medicinske vede 3.02 Klinična medicina

2.Sofinancerji

	Sofinancerji		
1.	Naziv	Ministrstvo za zdravje	
	Naslov	Štefanova 5, 1000 Ljubljana	

B. REZULTATI IN DOSEŽKI RAZISKOVALNEGA PROJEKTA

3.Povzetek raziskovalnega projekta[1]

SLO

Redke bolezni (RB) so posamič redke definiramo jih lahko s pojavnostjo manj kot 1 bolnik na 2000 (oz. 5 na 10.000) oseb, vendar je njihovo skupno število ocenjeno na preko 7000 bolezni, torej so kumulativo pogoste. Ocenjuje se, da ima kar 6-8 % ljudi eno od RB, kar pomeni, da je v Sloveniji po grobih ocenah okoli 150.000 bolnikov z RB. V 75 % se RB pojavljajo v otroštvu, v kar 30 % pa bolniki z RB preminejo pred 5. letom. V 80 % so RB genetskega izvora. Obravnava RB je zaradi njihove redkosti, genetske narave, prizadetosti več organskih sistemov in kroničnega poteka bolezni specifična; njihova nizka prevalenca zahteva specialistično obravnavo z visoko usposobljenimi strokovnjaki, specialno diagnostiko in z multidisciplinarnim pristopom, ki vključuje tudi psihološk o podporo, fizioterapijo in paliativno oskrbo s socialno podporo. Evropska komisija je ob zavedanju tega leta 2009 izdala priporočilo, ki terja od članic usmerjeno in organizirano obravnavo RB. Na tej podlagi je leta 2012 Ministrstvo za zdravje sprejelo Načrt dela na področju redkih bolezni v RS.

Za poučeno oblikovanje in sledenje učinkov zdravstvenih politik primarnega preprečevanja RB ter zdravstvene oskrbe bolnikov z RB, je nujno potrebno poznati epidemiološke podatke, vključno s podatki o zdravljenju in uporabi zdravil sirot. Registri predstavljajo ustrezen način za nepristransko zbiranje podatkov, spremljanje področja RB ter epidemiološke ali klinične raziskave in lahko v veliki meri pripomorejo k izboljšanju zdravstvenega varstva ter načrtovanja zdravstvene oskrbe bolnikov. Zaradi teh razlogov je razvoj registrov RB ena izmed prioritet na področju spremljanja in obvladovanja RB v EU, čemur pričajo tudi posebna priporočila in ukrepi za podporo razvoju tovrstnih registrov v evropskih zdravstvenih resolucijah in strateških dokumentih. Nacionalni register RB v Sloveniji doslej še ni bil vzpostavljen, je pa njegova vzpostavitev navedena med ostalimi ključnimi aktivnostmi v Načrtu in ena izmed nalog Akcijskega načrta za RB za leto 2015. V sklopu tega projekta smo razvili informacijsko in vsebinsko platformo ter zakonsko ureditev za nacionalni register redkih bolezni. Poleg tega smo razvili in uspešno implementirali modelni register redke bolezni - Nacionalni register družinske hiperholesterolemije in rekih dislipidemij. Za zgodnje odkrivanje pomembnega dela RB, ki spadajo med vrojene bolezni presnove (VBP) je ključen sistem presejanja novorojencev. V Sloveniji program presejanja novorojencev v času trajanja tega projekta vključuje le dve bolezni (fenilketonurijo in kongenitalni hipotiroidizem). V sklopu tega projekta smo razvili teoretsko podlago in natančen načrt širitve presejanja novorojencev in njene implementacije, ki jo načrtujemo v letu 2018.

ANG

Rare Diseases (RD) are individually rare – they are defined by the incidence of less than 1 patient in 2000 (e.g. 5 per 10,000 persons) but their total number is estimated at over 7,000 diseases, so they are frequent cumulatively. It is estimated that as many as 6-8% of the people have one of the RD, which means that in Slovenia, according to rough estimates, around 150,000 patients have a RD. In 75% the RD occur in childhood and in 30% the patients with RD pass away in 5 years. 80% of RD have a genetic origin. Treatment of RD is specific due to their rarity, genetic nature, multiorgan dysfunction and the chronic course of the disease; their low prevalence requires specialist treatment with highly qualified experts, special diagnostics and multidisciplinary approach that includes psychological support, physiotherapy and palliative care with social support. With that in mind the European Commission in 2009 issued a recommendation that requires the member states to direct and to organize RD treatment. On this basis, the Ministry of Health adopted a Plan of work in the field of rare diseases in the Republic of Slovenia in 2012.

For an educated formulation and tracking of the effects of health policies on primary prevention of RD and health care of patients with RD, it is necessary to know the epidemiological data, including information on the treatment and use of orphan medicinal products. Registries constitute an appropriate way of unbiased data collection, monitoring areas of RD and epidemiological or clinical research and can greatly contribute to improving health care and planning patient care. For these reasons, the development of registers of RD is one of the priorities in the area of surveillance and control of RD in the EU, to which the specific recommendations and measures to support the development of such registers in European health resolutions and strategic documents testify. National registry of RD in Slovenia has not yet been established, but its establishment is listed among other key activities in the Plan and one of the tasks of the Action Plan for RD in 2015. Within this research project we developed web-based platform and its legislational basis for National registry of RD. In addition, we successfully developed and implemented a model registry of rare disease - National registry of familial hypercholesterolemia and rare dyslipidemias. For early detection of a significant part of RD, which belong to the IMD, newborn screening is key. In Slovenia newborn screening program at the time of this project includes only two diseases (phenylketonuria and congenital hypothyroidism). Within this project we developed a theoretical background and an

specific action plan of implementation of extending the newborn screening , which will take place in 2018.

4.Poročilo o realizaciji predloženega programa dela oz. ciljev raziskovalnega projekta[2]

Pregled realizacije po glavnih zadanih ciljih, ki so bili izmed širše zadanih ciljev precizirani iz vidika njihove prioritetnosti:

- **Pilotski register redkih bolezni**

Projektna skupina je v sklopu predvidenih delovnih aktivnosti v letu 2016 natančneje opredelila temeljni namen registra, in sicer bi naj ta zagotavljal ustrezen nabor podatkov o redkih bolezni tako za statistične kot tudi za klinične potrebe. V ta namen je bila izvedena analiza osnovnega nabora skupnih podatkovnih elementov (common data elements - CDE) v EU projektih s tega področja (Parent, EpiRare) kot tudi pregled drugih nacionalnih registrov v državah EU..

V sklopu projekta smo nato v septembru 2017 uspeli uresničiti ta, glavni in prioritetni cilj projekta tj. vzpostavitev platforme nacionalnega registra redkih bolezni, v sodelovanju z zunanjim partnerjem podjetjem Marand. V tem času smo skupaj osnovali informacijsko platformo za nacionalni register redkih bolezni, oblikovano na podlagi OpenEHR, ki je odprta platforma. Oblikovali smo temeljni del registra redkih bolezni – t.i. nabora skupnih podatkovnih elementov (angl. »common data elements«), ki bodo skupni vsem posameznim redkim boleznim vključenim v nacionalni register; ta vsebuje 20 podatkov v 7 kategorijah. Naknadno smo po prejemu nabora podatkov EU RD PT ta nabor za namen priprave zakonskega predloga podlage delovanja registra redkih bolezni še prilagodili slednjemu, da bi bila na tej podlagi mogoča mednarodna izmenjava podatkov oz. sodelovanje v platformi EU RD PT. Naš nabor in nabor EU RD PT sta sicer kljub temu, da sta nastala neodvisno (naš nabor nekoliko prej) zelo primerljiva, brez večjih razlik, s tem smo dobili potrditev primernosti izbora nabora skupnih podatkovnih elementov, kot smo ga razvili v sklopu CRP. Nabor smo pripravili na podlagi naborov predlaganih v projektih Epirare, Parent CDE, italijanskega nacionalnega registra redkih bolezni, predloga Maranda. Z naborom skupnih podatkovnih elementov smo uspeli oblikovati t.i. epidemiološki nivo registra. Glede šifriranja bolezni, smo predvideli uporabo šifre diagnoze po MKB-10 (MKB-11) ter Orpha kode. Aktivnosti NIJZ za vzpostavitev pilotnega registra redkih bolezni v Republiki Sloveniji, ki je bil eden izmed najpomembnejših ciljev projekta so bile ključnega pomena za dokončno uresničitev vzpostavitve registra. V oktobru 2017 je bila spletna platforma za pilotski register redkih bolezni postavljena na strežnik NIJZ, s čemer bi bilo ob izpolnjevanju ustreznih pogojev (zakonska podlaga, celovito financiranje delovanja) lahko takoj pričeli z registracijo bolnikov.

V istem obdobju smo nadaljevali tudi z aktivnostmi priprave predloga naše delovne skupine za spremembo zakonodaje na tem področju, ki bi ga bilo mogoče vključiti v Zakon o zbirkah podatkov s področja zdravstva (ZZPPZ). Skladno s tem smo pripravili zakonsko podlago za njegovo delovanje v besedilu novele Zakona o zbirkah podatkov s področja zdravstvenega varstva (ZZPPZ, Uradni list RS, št. 65/00 in 47/15).

- **Nacionalni register družinske hiperholesterolemije in redkih dislipidemij**

V sklopu CRP smo se odločili, da smo kot primer »modelne« bolezni za razvoj kliničnega registra uporabili družinsko hiperholesterolemijo in redke dislipidemije. Sklado s tem, smo v sklopu projekta še v letu 2016 pričeli z razvijanjem prvega kliničnega registra redke bolezni - Nacionalnega registra družinske hiperholesterolemije in redkih dislipidemij, ki je v decembru 2016 uradno postal del evropske krovne zbirke podatkov na tem področju - "EAS Familial Hypercholesterolemia Study Collaboration". Informacijska platforma za Nacionalni register družinske hiperholesterolemije in redke dislipidemije je bila zatem dokončana v začetku leta 2017, s strani Komisije RS za medicinsko etiko je bila potrjena na januarski seji 2017. Konec marca 2017 smo začeli s prvimi vnosi v Register, ki je s tem že dokončno implementiran v prakso.

V Nacionalni register družinske hiperholesterolemije in redkih dislipidemij (Register) so vključene osebe, pri katerih bo diagnosticirana družinska hiperholesterolemija ali druga redka dislipidemija (oz. bo postavljen utemeljen klinični sum na eno od teh bolezni), in ki so po ustreznem pojasnilu podpisali (oz. pri mladoletnikih skladno z zakonodajo lahko njihovi skrbniki) privolitev za vključitev v Register. Za vključitev v Register za bolnike ni predvidena finančna nagrada. Skrbnika Registra sta Klinični oddelek za endokrinologijo, diabetes in bolezni presnove, Pediatrična klinika, UKC Ljubljana (predstojnik prof.

dr. Tadej Battelino) in Klinični oddelek za žilne bolezni, UKC Ljubljana (predstojnik prof. dr. Aleš Blinc); znotraj teh oddelkov so določeni zdravniki koordinatorji Registra ter predvidoma tudi drugi pooblaščeni sodelavci, skrbijo za pomoč pri tekočih vnosih v Register. V sodelovanju s firmo Marand je bila v sklopu tega projekta pripravljena spletna platforma za Register (ki obratuje na strežniku NIJZ), ki je močno olajšala zbiranje, varno hranjenje in izmenjavo podatkov zbranih v Registru in v katero lahko dostopajo koordinatorji Registra in druge pooblaščene osebe, ki bodo podatke tja vnašale. Spletno platformo za Register je računalniško podjetje Marand pripravilo, skladno z vsemi zahtevami za zbirke zdravstvenih podatkov.

Širitev programa presejanja novorojencev v Sloveniji in razvoj programa presejanja holesterola pri predšolskih otrocih

Širitev programa presejanja novorojencev

Posebno pozornost smo v tem obdobju posvetili širitvi programa presejanja novorojencev, ki je bil zastavljen kot eden od prioritetnih ciljev projekta. V maju 2016 smo uspešno oddali zadnjo verzijo vloge »Presejanje novorojencev za vrojene bolezni presnove s tandemsko masno spektrometrijo (MS/MS)« na Zdravstveni svet MZ. V sklopu tega programa smo predlagali in podrobno utemeljili potrebo po širitvi programa presejanja novorojencev iz obstoječih 2 na 16 redkih bolezni (ki smo jih izbrali glede na strokovne kriterije), ki smo jo deloma razvili tudi v sklopu tega projekta. Zdravstveni svet MZ je na seji dne 5. 10. 2016 dokončno odobril predlog širitve programa presejanja novorojencev, kar pomeni pomemben napredek na tem področju na nacionalnem nivoju. V letu 2018 načrtujemo uspešno implementacijo odobrenega programa presejanja v prakso. Na podlagi strokovnih kriterijev smo pripravili nabor bolezni, ki smo jih predlagali za širitev programa presejanja s tandemsko masno spektrometrijo. Za vsako izmed njih smo pripravili tudi natančen laboratorijski protokol izvedbe presejanja na podlagi lastnih rezultatov v sklopu pilotske študije na 10,000 novorojencih, ki je bila načrtovana in izvedena deloma v okviru CRP (članek Andraž Šmon et. al, Clin Biochem 2017).

Razvoj programa presejanja holesterola

Osebe z družinsko hiperholesterolemijo imajo izrazito, lahko tudi do 100-krat večje tveganje za zgodnji razvoj bolezni srca in ožilja, ki pogosto brez predhodnih opozoril prizadenejo ljudi v najbolj aktivnih letih življenja. Družinsko hiperholesterolemijo ima vsak petstoti posameznik v populaciji, po nekaterih novejših ocenah celo skoraj vsak dvestoti, kar pa pomeni, da ima homozigotno obliko družinske hiperholesterolemije okvirno 1/0,5 milijona, s čemer gre za bolezen, ki jo uvrščamo med zelo redke bolezni. V Sloveniji trenutno, kljub priporočilom nekaterih strokovnih forumov, še vedno kot edini v svetovnem merilu populacijsko presejamo hiperholesterolemijo pri predšolskih otrocih, ki jih zatem opredelimo še s pomočjo genetske analize, ki smo jo vpeljali na Pediatrični kliniki UKCL. Glede na naše analize s tem programom v zadnjih nekaj letih, ko je prišlo do dobre implementacije programa v prakso, že odkrivamo večino otrok z družinsko hiperholesterolemijo v populaciji. S pomočjo kaskadnega presejanja pa zatem tudi njihove starše, sorojence in druge svojce. Kohorta otrok, mladostnikov in mladih odraslih z družinsko hiperholesterolemijo, ki jih spremljamo v našem centru že sodi med večje v svetovnem merilu. Obravnava odraslih bolnikov z družinsko hiperholesterolemijo in redkimi dislipidemijami poteka na Kliniki za žilne bolezni, UKC Ljubljana, kamor so s Pediatrične klinike predani tudi odrasli pediatrični bolniki in svojci bolnikov, odkritih v sklopu kaskadnega presejanja. Doslej so bile na obeh omenjenih klinikah oblikovane že posamezne klinične zbirke podatkov, ki so vključevale bolnike z družinsko hiperholesterolemijo in redkimi dislipidemijami, ni pa še bil doslej vzpostavljen celovit in funkcionalen register bolnikov na nacionalni ravni. V sklopu CRP smo program presejanja holesterola revidirali na podlagi predhodnih rezultatov naše skupine, ter pripravili algoritem presejanja v obliki zgibanke, ki smo jo v avgustu 2017 že poslali vsem slovenskim pediatrom, ki presejanje holesterola pri predšloskih otrocih izvajajo.

Razvoj regionalne obravnave bolnikov z redko boleznijo

Ta del projekta je bil izvajan s strani partnerjev z Medicinske fakultete Univerze v Mariboru, v sodelovanju z UKC MB, ki so sodelovali tudi pri razvoju pilotskega registra redkih bolezni na nacionalni ravni.

V sodelovanju z UKC MB smo izgradili lokalni register bolnikov z družinsko hiperholesterolemijo, ki trenutno vključuje 102 bolnika. Za vse bolnike je izgrajena biobanka kliničnih vzorcev, ki vključuje DNA, RNA in proteine izolirane iz levkocitov periferne krvi ter eritrocite in serum. V dogovoru s UKC

LJ- Klinika za pediatrijo (prof. Tadej Batellino) bomo pričeli z izvajanjem genetskih analiz za znane gene. Za vzorce, ki bodo negativni za mutacije v znanih genih, bomo na MF UM izvedli analizo celotneg eksoma s tehnologijo sekvenciranja naslednje generacije (NGS). Bolniki se bodo lahko vključili v novi Nacioionalni register družinske hiperholesterolemije in redkih dislipidemij.;

V sodelovanju z UKC MB smo izgradli lokalni register za redke bolezni,, ki trenutno vključuje 29 bolnikov z redkimi boleznimi. Vzpostavljena je bila logistika in spremna dokumentacija za vključevanje bolnikov v lokalni register. Biobanka je opremljena s kliničnimi podatki urejenimi v skladu z razvitim minimalnim naborom podatkov (»common data set«) v okviru tega CRP projekta, tako da se bodo lahko vključili v novi Nacioionalni register za redke bolezni. Za vse bolnike je izgrajena biobanka kliničnih vzorcev, ki vključuje DNA, RNA in proteine izolirane iz levkocitov periferne krvi ter eritrocite in serum. Za del bolnikov smo že izvedli analizo celotneg eksoma s tehnologijo sekvenciranja naslednje generacije (NGS) in nekatere mutacije potrdili s standardno metodo sekvenciranja po Sangerju in z metodo RFLP.

Izvedli smo analizo stanja obravnave redkih bolezni v SV regiji.

Ostali cilji CRP

Na področju modelne redke bolezni – Fabry-jeve bolezni so bile v tem obdobju izvedene prirpave za vključitev teh bolnikov v register RB, z urejanjem baz podatkov, posodobitev in uskladitev nomenklature mutacij z mednarodnimi priporočili, izdelan je bil predlog privolitve bolnika pred vpisom v register.

Del informacij o redkih boleznih je dostopen tudi v okviru nacionalne strani Orphanet Slovenija, kjer pripravljamo posodobitev in dodatek novih informacij V okviru izobraževanja zdravnikov primarnega nivoja, bodočih specialistov družinske medicine, smo aktivno udeleženi s predstavitvijo področja redkih bolezni in njihove diagnostike, kjer smo zaznali pomembne izzive pri predajanju informacij in posodabljajnu znanja. V ta namen je že v pripravi predlog modularnih informacij in pripravo materialov vezanih na področje redkih bolezni za strokovno javnost.

5. Ocena stopnje realizacije programa dela na raziskovalnem projektu in zastavljenih raziskovalnih ciljev[3]

Stopnja realizacije glavnih ciljev projekta, ki so bili zadani skladno s postavljenimi prioritetami (tj. podlage in zasnova za nacionalni register redkih bolezni, oblikovanje registra modelne skupine redkih bolezni, priprava programa razširjenega presejanja novorojencev, razvoj regionalne obravnave redkih bolezni) je bila v celoti skladna z načrtom oz. je tega v nekaterih vidikih celo presegala. Zadane prioritetne cilje v sklopu projekta smo s tem uspeli uresničiti.

6. Spremembe programa dela raziskovalnega projekta oziroma spremembe sestave projektne skupine[4]

Brez sprememb programa raziskovalnega projekta oziroma sestave projektne skupine.

7. Najpomembnejši dosežki projektne skupine na raziskovalnem področju[5]

	Dosežek			
1.	COBISS ID		4473260	Vir: COBISS.SI
	Naslov	*SLO*	Sekveniranje nove generacije kot potrditveni test razširjenega presejanja novorojence.	
		ANG	Next generation sequencing as a follow-up test in an expanded newborn screening programme	
	Opis	*SLO*	Raziskava pilotnega projekta razširjenega presejanja novorojencev na 10,000 novorojencih. Raziskava je omogočila pripravo podlag za širitev presejanja novorojencev.	

	Dosežek		
		ANG	Study of pilot project of expanded newborn screening programme in 10,000 newborns. The study was a basis for successful implementation of expanded newborn screening program.
	Objavljeno v		Elsevier.; Clinical biochemistry; 2018; Vol. 52; str. 48-55; Impact Factor: 2.434;Srednja vrednost revije / Medium Category Impact Factor: 2.314; WoS: PW; Avtorji / Authors: Šmon Andraž, Repič-Lampret Barbka, Grošelj Urh, Žerjav-Tanšek Mojca, Kovač Jernej, Perko Daša, Bertok Sara, Battelino Tadej, Trebušak Podkrajšek Katarina
	Tipologija		1.01 Izvirni znanstveni članek
2.	COBISS ID		4610476 Vir: COBISS.SI
	Naslov	*SLO*	MCADD - Dve novi mutaciji gena ACADM s pomočjo retrogradnega presejanja
		ANG	Medium-chain acyl-CoA dehydrogenase deficiency: Two novel ACADM mutations identified in a retrospective screening.
	Opis	*SLO*	Raziskava v sklopu pilotskega projekta razširjenega presejanja
		ANG	Study in the context of pilot project of expanded newborn screening
	Objavljeno v		Cambridge Medical Publications Ltd; Journal of international medical research; 2018; Vol. 46, no.; str. 1-10; Impact Factor: 1.323;Srednja vrednost revije / Medium Category Impact Factor: 3.175; WoS: QA, TU; Avtorji / Authors: Šmon Andraž, Grošelj Urh, Debeljak Maruša, Žerjav-Tanšek Mojca, Bertok Sara, Avbelj Magdalena, Trebušak Podkrajšek Katarina, Battelino Tadej, Repič-Lampret Barbka
	Tipologija		1.02 Pregledni znanstveni članek
3.	COBISS ID		4156901 Vir: COBISS.SI
	Naslov	*SLO*	Register redkih bolezni
		ANG	Rare disease registry
	Opis	*SLO*	Pregled področja registrov redkih bolezni in ustreznih informacijskih podlag.
		ANG	Review article of rare disease registries and appropriate informational platforms
	Objavljeno v		Zdravstveni dom dr. Adolfa Drolca; Informatica medica slovenica; 2017; Letn. 22, št. 1/2; str. 9-21; Avtorji / Authors: Stanimirović Dalibor, Murko Eva
	Tipologija		1.01 Izvirni znanstveni članek
4.	COBISS ID		5089454 Vir: COBISS.SI
	Naslov	*SLO*	Interoperabilnost EHR
		ANG	Special topic interoperability and EHR
	Opis	*SLO*	Pregled informacijske tehnologije za razvoj registra redkih bolezni
		ANG	Review of IT for development of rare diseases registry
	Objavljeno v		Schattauer; Applied clinical informatics; 2017; Vol. 8, iss. 3; Impact Factor: 1.496;Srednja vrednost revije / Medium Category Impact Factor: 2.287; WoS: PT; Avtorji / Authors: Beštek Mate, Stanimirović Dalibor
	Tipologija		1.01 Izvirni znanstveni članek

8.Najpomembnejši dosežek projektne skupine na področju gospodarstva, družbenih in kulturnih dejavnosti[6]

	Dosežek

	Dosežek			
1.	COBISS ID			
	Naslov	*SLO*		
		ANG		
	Opis	*SLO*		
		ANG		
	Šifra			
	Objavljeno v			
	Tipologija			

9.Drugi pomembni rezultati projektne skupine[7]

GROŠELJ, Urh, BATTELINO, Tadej, KOVAČ, Jernej, ŠIRCA-ČAMPA, Andreja. Nacionalni program presejalnega testiranja za holesterol pri otrocih : algoritem za izvajanje programa presejanja za holesterol pri predšolskih otrocih in prehranske smernice. Ljubljana: Klinični oddelek za endokrinologijo, diabetes in bolezni presnove, Služba za dietoterapijo in bolniško prehrano in Služba za specialno laboratorijsko diagnostiko Pediatrične klinike UKC, 2017. 4 str. [COBISS.SI-ID 4071852] MURKO, Eva, STANIMIROVIĆ, Dalibor, GROŠELJ, Urh. Register redkih bolezni - (trenutna) stopnja razvoja. V: SLAPAR, Majda (ur.). Dan redkih bolezni, 28. februar 2018 : z raziskavami so možnosti neomejene. 8. izd. Mengeš: Društvo bolnikov s krvnimi boleznimi Slovenije. 2018, str. 19-22. http://www.drustvo-bkb.si/media/moddoc_20_c950e4c2fb5e2a2825f008.pdf. [COBISS.SI-ID 4170725] ŽERJAV-TANŠEK, Mojca, GROŠELJ, Urh, BATTELINO, Tadej. Redke bolezni-primer prirojeni presnovnih bolezni. V: Dan redkih bolezni 2017, 28. februar : z raziskavami so možnosti neomejene. 7. izd. Mengeš: Društvo bolnikov s krvnimi boleznimi. 2017, str. 12-20. [COBISS.SI-ID 3645356]

10.Pomen raziskovalnih rezultatov projektne skupine[8]

10.1. Pomen za razvoj znanosti[9]

SLO

V sklopu projekta smo uspeli uresničiti več ciljev, ki so pomembni za razvoj znanosti: - Razvoj platforme (vsebinsko, tehnološko, zakonska ureditev) za uspešno implementacijo nacionalnega registra redkih bolezni, ki bo ob uresničitvi sodil med najnaprednejše tovrstne zbirke podatkov v mednarodnem merilu in bo pomembno izhodišče za raziskovanje redkih bolezni. - Razvoj in uspešna implementacija modelnega registra redke bolezni "Nacionalni register družinske hiperholesterolemije in redkih dislipidemij" in njegova uspešna implementacija v krovni mednarodni register na tem področju EAS FHSC, ki pomeni pomembno izhodišče za raziskovanje te skupine bolezni v mednarodnem merilu. - Razvoj strokovnih in organizacijskih podlag za izvedbo širitve presejanja novorojencev in izvedba pilotske raziskave na 10,000 novorojencih z uporabo NGS metode kot potrditvenega testiranja. Nadaljnji razvoj univerzalnega presejanja holesterola pri predšolskih otrocih, ki je edinstven tovrstni program v svetovnem merilu. Oboje ima velik pomen za znanstveni razvoj in nadaljnje raziskave na področju presejalnih testiranj.

ANG

Within this project, several aims were realized which are important for advancing science: - Development of platform (content, technology, legislation) for a successful implementation of national registry of rare diseases, which will be when implemented one of most advanced such registries in the international context and will present an important starting point for research of rare diseases. - Development and successful implementation of a model disease registry of a rare disease - "National registry of familial hypercholesterolemia and rare dyslipidemias" and its successful implementation into the main internaitional registry in the field EAS FHSC, which is an important starting point for research activities in the international context. - Development of professional and organizational bases for implementation of expanded newborn screening in Slovenia and completion of a pilot research study on 10,000 newborns where NGS genetic method was used as a follow up

test. Further development of universal cholesterol screening in pre-school children, which is a unique program internationally. Both is of a high scientific importance.

10.2. Pomen za razvoj Slovenije[10]

SLO

V sklopu projekta smo uspeli uresničiti več ciljev, ki so pomembni za razvoj Slovenije: - Razvoj platforme (vsebinsko, tehnološko, zakonska ureditev) za nacionalni register redkih bolezni, ki bo ob uresničitvi sodil med najnaprednejše tovrstne zbirke podatkov v mednarodnem merilu in bo pomemben tudi za razvoj Slovenije na tem področju v mednarodnem merilu in za kakovostno obravnavo bolnikov z redkimi boleznimi v Sloveniji. - Razvoj in uspešna implementacija modelnega registra redke bolezni "Nacionalni register družinske hiperholesterolemije in redkih dislipidemij" in njegova uspešna implementacija v krovni mednarodni register na tem področju EAS FHSC, ki pomeni pomembno izhodišče za razvoj Slovenije v mednarodnem merilu in za zagotavljanje najboljše možne oskrbe za slovenske bolnike. - Razvoj strokovnih in organizacijskih podlag za izvedbo širitve presejanja novorojencev in izvedba pilotske raziskave na 10,000 novorojencih, vključno z uporabo najnovejše genetske tehnologije (NGS) kot potrditvenega testa. Nadaljnji razvoj univerzalnega presejanja holesterola pri predšolskih otrocih, ki je edinstven tovrstni program v svetovnem merilu. Oboje ima velik razvojni in zdravstveni pomen za Slovenijo in njene prebivalce.

ANG

Within this project, several aims were realized which are important for development of Slovenia: - Development of platform (content, technology, legislation) for a successful implementation of national registry of rare diseases, which will be when implemented one of most advanced such registries in the international context and will present an important starting point for research of rare diseases and for providing a quality health care for patients with rare diseases. - Development and successful implementation of a model disease registry of a rare disease - "National registry of familial hypercholesterolemia and rare dyslipidemias" and its successful implementation into the main internaitional registry in the field EAS FHSC, which is important also for development of Slovenia in this particular field and for providing the best possible care for Sloveniana patients. - Development of professional and organizational bases for implementation of expanded newborn screening in Slovenia and completion of a pilot research study on 10,000 newborns where NGS genetic method was used as a follow up test. Further development of universal cholesterol screening in pre-school children, which is a unique program internationally. Both is of a high importance for health care system in Slovenia and health care of its citizens.

11.Vpetost raziskovalnih rezultatov projektne skupine

11.1. Vpetost raziskave v domače okolje

Kje obstaja verjetnost, da bodo vaša znanstvena spoznanja deležna zaznavnega odziva?

1 v domačih znanstvenih krogih

2 pri domačih uporabnikih

Kdo (poleg sofinancerjev) že izraža interes po vaših spoznanjih oziroma rezultatih?[1] [1]

Rezultati raziskav izvedenih v sklopu tega projekta, ki so bili publicirani v mednarodni znanstveni literaturi ali predstavljeni na mednarodnih srečanjih so bili deležni interesa različnih drugih raziskovalnih skupin ali forumov, z nekaterimi načrtujemo sodelovanje v prihodnosti.

11.2. Vpetost raziskave v tuje okolje

Kje obstaja verjetnost, da bodo vaša znanstvena spoznanja deležna zaznavnega odziva?

1 v mednarodnih znanstvenih krogih

2 pri mednarodnih uporabnikih

Navedite število in obliko formalnega raziskovalnega sodelovanja s tujini raziskovalnimi inštitucijami:[1] [2]

V času trajanja projekta sta se dva izmed sodelujočih centrov (Pediatrična klinika in pa Klinični inštitut za medicinsko genetiko UKC Ljubljana) uspešno vključila v novonastale evropske referenčne mreže za redke bolezni - European Reference Networks (ERN), znotraj teh v mrežo za redke presnovne bolezni, redke endokrine bolezni, redke bolezni živčevja in redke živčno-mišične bolezni.

Kateri so rezultati tovrstnega sodelovanja:[1] [3]

Sodelovanje v referenčnih mrežah za redke bolezni (ERN) omogoča strokovno in znanstveno sodelovanje, izmenjavo izkušenj in skupni razvoj področja, z najuglednejšimi centri v Evropi za posamezna podpodročja.

12. Označite, katerega od navedenih ciljev ste si zastavili pri projektu, katere konkretne rezultate ste dosegli in v kakšni meri so doseženi rezultati uporabljeni

Cilj		
F.01	**Pridobitev novih praktičnih znanj, informacij in veščin**	
	Zastavljen cilj	DA DA NE NE
	Rezultat	Dosežen
	Uporaba rezultatov	Uporabljen bo v naslednjih 3 letih
F.02	**Pridobitev novih znanstvenih spoznanj**	
	Zastavljen cilj	DA DA NE NE
	Rezultat	Dosežen
	Uporaba rezultatov	Uporabljen bo v naslednjih 3 letih
F.03	**Večja usposobljenost raziskovalno-razvojnega osebja**	
	Zastavljen cilj	DA DA NE NE
	Rezultat	Dosežen
	Uporaba rezultatov	Uporabljen bo v naslednjih 3 letih
F.04	**Dvig tehnološke ravni**	
	Zastavljen cilj	DA DA NE NE
	Rezultat	Dosežen
	Uporaba rezultatov	Uporabljen bo v naslednjih 3 letih
F.05	**Sposobnost za začetek novega tehnološkega razvoja**	
	Zastavljen cilj	DA DA NE NE
	Rezultat	
	Uporaba rezultatov	
F.06	**Razvoj novega izdelka**	
	Zastavljen cilj	DA DA NE NE
	Rezultat	
	Uporaba rezultatov	
F.07	**Izboljšanje obstoječega izdelka**	

	Zastavljen cilj	DA DA NE NE
	Rezultat	
	Uporaba rezultatov	
F.08	**Razvoj in izdelava prototipa**	
	Zastavljen cilj	DA DA NE NE
	Rezultat	
	Uporaba rezultatov	
F.09	**Razvoj novega tehnološkega procesa oz. tehnologije**	
	Zastavljen cilj	DA DA NE NE
	Rezultat	
	Uporaba rezultatov	
F.10	**Izboljšanje obstoječega tehnološkega procesa oz. tehnologije**	
	Zastavljen cilj	DA DA NE NE
	Rezultat	
	Uporaba rezultatov	
F.11	**Razvoj nove storitve**	
	Zastavljen cilj	DA DA NE NE
	Rezultat	Dosežen
	Uporaba rezultatov	Uporabljen bo v naslednjih 3 letih
F.12	**Izboljšanje obstoječe storitve**	
	Zastavljen cilj	DA DA NE NE
	Rezultat	
	Uporaba rezultatov	
F.13	**Razvoj novih proizvodnih metod in instrumentov oz. proizvodnih procesov**	
	Zastavljen cilj	DA DA NE NE
	Rezultat	
	Uporaba rezultatov	
F.14	**Izboljšanje obstoječih proizvodnih metod in instrumentov oz. proizvodnih procesov**	
	Zastavljen cilj	DA DA NE NE
	Rezultat	
	Uporaba rezultatov	
F.15	**Razvoj novega informacijskega sistema/podatkovnih baz**	
	Zastavljen cilj	DA DA NE NE
	Rezultat	Dosežen
	Uporaba rezultatov	Uporabljen bo v naslednjih 3 letih
F.16	**Izboljšanje obstoječega informacijskega sistema/podatkovnih baz**	
	Zastavljen cilj	DA DA NE NE

	Rezultat	
	Uporaba rezultatov	
F.17	**Prenos obstoječih tehnologij, znanj, metod in postopkov v prakso**	
	Zastavljen cilj	DA DA NE NE
	Rezultat	
	Uporaba rezultatov	
F.18	**Posredovanje novih znanj neposrednim uporabnikom (seminarji, forumi, konference)**	
	Zastavljen cilj	DA DA NE NE
	Rezultat	Dosežen
	Uporaba rezultatov	V celoti
F.19	**Znanje, ki vodi k ustanovitvi novega podjetja ("spin off")**	
	Zastavljen cilj	DA DA NE NE
	Rezultat	
	Uporaba rezultatov	
F.20	**Ustanovitev novega podjetja ("spin off")**	
	Zastavljen cilj	DA DA NE NE
	Rezultat	
	Uporaba rezultatov	
F.21	**Razvoj novih zdravstvenih/diagnostičnih metod/postopkov**	
	Zastavljen cilj	DA DA NE NE
	Rezultat	Dosežen
	Uporaba rezultatov	V celoti
F.22	**Izboljšanje obstoječih zdravstvenih/diagnostičnih metod/postopkov**	
	Zastavljen cilj	DA DA NE NE
	Rezultat	Dosežen
	Uporaba rezultatov	V celoti
F.23	**Razvoj novih sistemskih, normativnih, programskih in metodoloških rešitev**	
	Zastavljen cilj	DA DA NE NE
	Rezultat	
	Uporaba rezultatov	
F.24	**Izboljšanje obstoječih sistemskih, normativnih, programskih in metodoloških rešitev**	
	Zastavljen cilj	DA DA NE NE
	Rezultat	
	Uporaba rezultatov	
F.25	**Razvoj novih organizacijskih in upravljavskih rešitev**	
	Zastavljen cilj	DA DA NE NE

	Rezultat	Dosežen
	Uporaba rezultatov	Uporabljen bo v naslednjih 3 letih
F.26	**Izboljšanje obstoječih organizacijskih in upravljavskih rešitev**	
	Zastavljen cilj	DA DA NE NE
	Rezultat	
	Uporaba rezultatov	
F.27	**Prispevek k ohranjanju/varovanje naravne in kulturne dediščine**	
	Zastavljen cilj	DA DA NE NE
	Rezultat	
	Uporaba rezultatov	
F.28	**Priprava/organizacija razstave**	
	Zastavljen cilj	DA DA NE NE
	Rezultat	
	Uporaba rezultatov	
F.29	**Prispevek k razvoju nacionalne kulturne identitete**	
	Zastavljen cilj	DA DA NE NE
	Rezultat	
	Uporaba rezultatov	
F.30	**Strokovna ocena stanja**	
	Zastavljen cilj	DA DA NE NE
	Rezultat	Dosežen
	Uporaba rezultatov	V celoti
F.31	**Razvoj standardov**	
	Zastavljen cilj	DA DA NE NE
	Rezultat	Dosežen
	Uporaba rezultatov	V celoti
F.32	**Mednarodni patent**	
	Zastavljen cilj	DA DA NE NE
	Rezultat	
	Uporaba rezultatov	
F.33	**Patent v Sloveniji**	
	Zastavljen cilj	DA DA NE NE
	Rezultat	
	Uporaba rezultatov	
F.34	**Svetovalna dejavnost**	
	Zastavljen cilj	DA DA NE NE
	Rezultat	
	Uporaba rezultatov	

F.35	**Drugo**	
	Zastavljen cilj	DA DA NE NE
	Rezultat	
	Uporaba rezultatov	

Komentar

13.Označite potencialne vplive oziroma učinke vaših rezultatov na navedena področja

	Vpliv	**Ni vpliva**	**Majhen vpliv**	**Srednji vpliv**	**Velik vpliv**	
G.01	**Razvoj visokošolskega izobraževanja**					
G.01.01.	Razvoj dodiplomskega izobraževanja	1	2	3	4	
G.01.02.	Razvoj podiplomskega izobraževanja	1	2	3	4	
G.01.03.	Drugo:	1	2	3	4	
G.02	**Gospodarski razvoj**					
G.02.01	Razširitev ponudbe novih izdelkov/storitev na trgu	1	2	3	4	
G.02.02.	Širitev obstoječih trgov	1	2	3	4	
G.02.03.	Znižanje stroškov proizvodnje	1	2	3	4	
G.02.04.	Zmanjšanje porabe materialov in energije	1	2	3	4	
G.02.05.	Razširitev področja dejavnosti	1	2	3	4	
G.02.06.	Večja konkurenčna sposobnost	1	2	3	4	
G.02.07.	Večji delež izvoza	1	2	3	4	
G.02.08.	Povečanje dobička	1	2	3	4	
G.02.09.	Nova delovna mesta	1	2	3	4	
G.02.10.	Dvig izobrazbene strukture zaposlenih	1	2	3	4	
G.02.11.	Nov investicijski zagon	1	2	3	4	
G.02.12.	Drugo:	1	2	3	4	
G.03	**Tehnološki razvoj**					
G.03.01.	Tehnološka razširitev/posodobitev dejavnosti	1	2	3	4	
G.03.02.	Tehnološko prestrukturiranje dejavnosti	1	2	3	4	
G.03.03.	Uvajanje novih tehnologij	1	2	3	4	
G.03.04.	Drugo:	1	2	3	4	
G.04	**Družbeni razvoj**					
G.04.01	Dvig kvalitete življenja	1	2	3	4	
G.04.02.	Izboljšanje vodenja in upravljanja	1	2	3	4	
G.04.03.	Izboljšanje delovanja administracije in javne uprave	1	2	3	4	
G.04.04.	Razvoj socialnih dejavnosti	1	2	3	4	

G.04.05.	Razvoj civilne družbe	1	2	3	4	
G.04.06.	Drugo:	1	2	3	4	
G.05.	**Ohranjanje in razvoj nacionalne naravne in kulturne dediščine in identitete**	1	2	3	4	
G.06.	**Varovanje okolja in trajnostni razvoj**	1	2	3	4	
G.07	**Razvoj družbene infrastrukture**					
G.07.01.	Informacijsko-komunikacijska infrastruktura	1	2	3	4	
G.07.02.	Prometna infrastruktura	1	2	3	4	
G.07.03.	Energetska infrastruktura	1	2	3	4	
G.07.04.	Drugo:	1	2	3	4	
G.08.	**Varovanje zdravja in razvoj zdravstvenega varstva**	1	2	3	4	
G.09.	**Drugo:**	1	2	3	4	

Komentar

14. Naslov spletne strani za projekte, odobrene na podlagi javnih razpisov za sofinanciranje raziskovalnih projektov za leti 2015 in 2016[14]

Projekt je predstavljen na spletni strani Nacionalne kontaktne točke za redke bolezni: http://www.redkebolezni.si/splosno/slovenske-in-mednarodne-raziskave-o-redkih-boleznih-ki-trenutno-potekajo-v-sloveniji/

C. IZJAVE

Podpisani izjavljam/o, da:
- so vsi podatki, ki jih navajamo v poročilu, resnični in točni;
- se strinjamo z obdelavo podatkov v skladu z zakonodajo o varstvu osebnih podatkov za potrebe ocenjevanja in obdelavo teh podatkov za evidence ARRS;
- so vsi podatki v obrazcu v elektronski obliki identični podatkom v obrazcu v pisni obliki (v primeru, da poročilo ne bo oddano z digitalnima podpisoma);
- so z vsebino zaključnega poročila seznanjeni in se strinjajo vsi soizvajalci projekta;
- bomo sofinancerjem istočasno z zaključnim poročilom predložili tudi elaborat na zgoščenki (CD), ki ga bomo posredovali po pošti, skladno z zahtevami sofinancerjev.

Podpisi:

zastopnik oz. pooblaščena oseba raziskovalne organizacije:	in	*vodja raziskovalnega projekta:*
Univerzitetni klinični center Ljubljana		Tadej Battelino

ŽIG

Datum: 15.3.2018

Oznaka poročila: ARRS-CRP-ZP-2018/42

[1] Napišite povzetek raziskovalnega projekta (največ 3.000 znakov v slovenskem in angleškem jeziku). Nazaj

[2] Navedite cilje iz prijave projekta in napišite, ali so bili cilji projekta doseženi. Navedite ključne ugotovitve, znanstvena spoznanja, rezultate in učinke raziskovalnega projekta in njihovo uporabo ter sodelovanje s tujimi partnerji. Največ 12.000 znakov vključno s presledki (približno dve strani, velikost pisave 11). Nazaj

[3] Realizacija raziskovalne hipoteze. Največ 3.000 znakov vključno s presledki (približno pol strani, velikost pisave 11). Nazaj

[4] Navedite morebitna bistvena odstopanja in spremembe od predvidenega programa dela raziskovalnega projekta, zapisanega v prijavi raziskovalnega projekta. Navedite in utemeljite tudi spremembe sestave projektne skupine v zadnjem letu izvajanja projekta (t. j. v letu 2016). . Če sprememb ni bilo, navedite »Ni bilo sprememb«. Največ 6.000 znakov vključno s presledki (približno ena stran, velikosti pisave 11). Nazaj

[5] Navedite dosežke na raziskovalnem področju (največ deset), ki so nastali v okviru tega projekta.

Raziskovalni dosežek iz obdobja izvajanja projekta (do oddaje zaključnega poročila) vpišete tako, da izpolnite COBISS kodo dosežka – sistem nato sam izpolni naslov objave, naziv, IF in srednjo vrednost revije, naziv FOS področja ter podatek, ali je dosežek uvrščen v A'' ali A'. Nazaj

[6] Navedite dosežke na področju gospodarstva, družbenih in kulturnih dejavnosti (največ pet), ki so nastali v okviru tega projekta.

Dosežek iz obdobja izvajanja projekta (do oddaje zaključnega poročila) vpišete tako, da izpolnite COBISS kodo dosežka, sistem nato sam izpolni podatke, manjkajoče rubrike o dosežku pa izpolnite.

Dosežek na področju gospodarstva, družbenih in kulturnih dejavnosti je po svoji strukturi drugačen kot znanstveni dosežek. Povzetek znanstvenega dosežka je praviloma povzetek bibliografske enote (članka, knjige), v kateri je dosežek objavljen.

Povzetek dosežka na področju gospodarstva, družbenih in kulturnih dejavnosti praviloma ni povzetek bibliografske enote, ki ta dosežek dokumentira, ker je dosežek sklop več rezultatov raziskovanja, ki je lahko dokumentiran v različnih bibliografskih enotah. COBISS ID zato ni enoznačen izjemoma pa ga lahko tudi ni (npr. prehod mlajših sodelavcev v gospodarstvo na pomembnih raziskovalnih nalogah, ali ustanovitev podjetja kot rezultat projekta … - v obeh primerih ni COBISS ID). Nazaj

[7] Navedite rezultate raziskovalnega projekta iz obdobja izvajanja projekta (do oddaje zaključnega poročila) v primeru, da katerega od rezultatov ni mogoče navesti v točkah 7 in 8 (npr. v sistemu COBISS rezultat ni evidentiran). Največ 2.000 znakov, vključno s presledki. Nazaj

[8] Pomen raziskovalnih rezultatov za razvoj znanosti in za razvoj Slovenije bo objavljen na spletni strani: http://sicris.izum.si/ za posamezen projekt, ki je predmet poročanja. Nazaj

[9] Največ 4.000 znakov, vključno s presledki. Nazaj

[10] Največ 4.000 znakov, vključno s presledki. Nazaj

[11] Največ 500 znakov, vključno s presledki. Nazaj

[12] Največ 500 znakov, vključno s presledki. Nazaj

[13] Največ 1.000 znakov, vključno s presledki. Nazaj

[14] Izvajalec mora za projekte, odobrene na podlagi Javnega razpisa za izbiro raziskovalnih projektov Ciljnega raziskovalnega programa »CRP 2016« v letu 2016 in Javnega razpisa za izbiro raziskovalnih projektov Ciljnega raziskovalnega programa »Zagotovimo.si hrano za jutri« v letu 2016, na spletnem mestu svoje RO odpreti posebno spletno stran, ki je namenjena projektu. Obvezne vsebine spletne strani so: vsebinski opis projekta z osnovnimi podatki glede financiranja, sestava projektne skupine s povezavami na SICRIS, faze projekta in njihova realizacija, bibliografske reference, ki izhajajo neposredno iz izvajanja projekta ter logotip ARRS in drugih sofinancerjev. Spletna stran mora ostati aktivna še 5 let po zaključku projekta. Nazaj

Obrazec: ARRS-CRP-ZP/2018 v1.00
54-C8-2B-8C-D3-A3-30-AE-C3-43-90-ED-0C-88-61-19-2F-B3-DB-EF

Priloga 1. REGISTER REDKIH BOLEZNI – NABOR PODATKOV

i.	**Identifikatorji** 1. Naziv registra, šifra 2. EMŠO, KZZ, MI (Marand) 3. Datum registracije 4. BPI zdravnika (Marand) 5. Identifikator/ime bolnišnice **6.** Značilnosti privolitve bolnika (za register/raziskave/vzorci/tujina)
ii.	**Demografski podatki** 7. Ime in priimek 8. Datum rojstva (DD/MM/LLLL) 9. Spol (M/Ž/N) 10. Naslov prebivališče 11. Kraj rojstva (mesto, država) **12.** Živ/mrtev (datum smrti; – se generira iz CRP)
iii.	**Klinični podatki** 13. Šifra diagnoze(MKB10 , OrphaID) 14. Ime diagnoze (+možnost dodatnega opisa dg) 15. Potrjena? DA/VERJETNA 16. Datum diagnoze (starost ob diagnozi) 17. Čas nastopa simptomov (leto)
iv.	**Genetski podatki in biološki material** 18. Za vsako od ugotovljenih različic: Ime gena z ugotovljeno različico (po HGNC nomenklaturi) ali kromosoma **19.** Biološki material (NA VOLJO, KATERI in KJE)
V.	**Terapevtski sklop** **20.** Ali ima zdravilo sirota glede na iskalnik EMA

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE
Directorate F – Health, Consumers and Reference Materials
Unit F.1 – Health in Society

EUROPEAN PLATFORM ON RARE DISEASES REGISTRATION (EU RD Platform)

SET OF COMMON DATA ELEMENTS FOR RARE DISEASES REGISTRATION

GROUP	ELEMENT N°	ELEMENT NAME	ELEMENT DESCRIPTION	CODING	COMMENT
1. Pseudonym	1.1.	Pseudonym	Patient's pseudonym	• String	The JRC is working on providing a pseudonymisation tool to the registries
2. Personal information	2.1.	Date of birth	Patient's date of birth	• Date (dd/mm/yyyy)	
	2.2.	Sex	Patient's sex at birth	• Female • Male • Undetermined • Foetus (Unknown)	
3. Patient Status	3.1.	Patient's status	Patient alive or dead	• Alive • Dead • Lost in follow-up • Opted-out	If dead then answer question 3.2
	3.2.	Date of death	Patient's date of death	• Date (dd/mm/yyyy)	
4. Care pathway	4.1.	First contact with specialised centre	Date of first contact with specialised centre	• Date (dd/mm/yyyy)	

5. Disease history	5.1.	Age at onset	Age at which symptoms/signs first appeared	• Antenatal • At birth • Date (dd/mm/yyyy) • Undetermined	
	5.2.	Age at diagnosis	Age at which diagnosis was made	• Antenatal • At birth • Date (dd/mm/yyyy) • Undetermined	
6 Diagnosis	6.1.	Diagnosis of the rare disease	Diagnosis retained by the specialised centre	Orpha code (strongly recommended – see link) / Alpha code/ ICD-9 code/ ICD-9-CM code / ICD-10 code	http://www.orphadata.org/cgi-bin/inc/product1.inc.php
	6.2.	Genetic diagnosis	Genetic diagnosis retained by the specialised centre	International classification of mutations (HGVS) (strongly recommended – see link) / HGNC / OMIM code	http://www.hgvs.org
	6.3	Undiagnosed case	How the undiagnosed case is defined	• Phenotype (HPO) • Genotype (HGVS)	
7. Research	7.1.	Agreement to be contacted for research purposes	Patient's permission exists for being contacted for research purposes	• YES • NO	
	7.2.	Consent to the reuse of data	Patient's consent exists for his/her data to be reused for other research purposes	• YES • NO	
	7.3.	Biological sample	Patient's biological sample available for research	• YES • NO	If YES answer question 7.4
	7.4.	Link to a biobank	Biological sample stored in a biobank	• YES (if appropriate use link) • NO	https://directory.bbmri-eric.eu
8.Disability	8.1.	Classification of functioning/disability	Patient's disability profile according to International Classification of Functioning and Disability (ICF)	• Disability profile / Score	http://www.who.int/classifications/icf/whodasii/en/

ZAKON O ZBIRKAH PODATKOV S PODRO ČJA ZDRAVSTVENEGA VARSTVA (ZZPPZ-1)

PRILOGA

Ime zbirke	**1. Register redkih bolezni Republike Slovenije**
Namen zbiranja	Register redkih bolezni Republike Slovenije se vodi z namenom obdelovanja podatkov o incidenci, prevalenci in preživetju bolnikov z redko boleznijo, spremljanja, načrtovanja in vrednotenja zdravstvenega varstva, ter kot osnova za epidemiološke in klinične raziskave.
Zbirka	1.1 Register redkih bolezni Republike Slovenije
Vsebina podatkov v zbirki	**Skupina obveznih podatkov** **i. Identifikatorji** 1. Naziv registra (če obstaja znotraj krovnega registra redkih bolezni), šifra 2. EMŠO, KZZ 3. Datum registracije 4. Datum prvega stika s specialističnim centrom 5. Številka zdravnika, ki je bolnika registriral 6. Identifikator/ime bolnišnice 7. Značilnosti privolitve bolnika (Za vključitev v register/Za vključitev v raziskave/Za uporabo bioloških vzorcev/Pošiljanje podatkov v tujino/Pošiljanje vzorcev v tujino) **ii. Demografski podatki** 8. Ime in priimek 9. Datum rojstva (DD/MM/LLLL) 10. Spol ob rojstvu (M/Ž/nedoločen/neznano) 11. Naslov prebivališča (stalno in za časno, po šifrantu prostorskih enot in ulic Registra prostorskih enot) 12. Kraj rojstva (mesto, država) 13. Živ/Mrtev (datum smrti)/Izgubljen/Izločen **iii. Klinični podatki** 14. Šifra diagnoze po MKB10 in Orpha kodah 15. Ime diagnoze z možnostjo dodatnega opisa dg 16. Podatek o potrjenosti diagnoze (DA/VERJETNA) 17. Opis nediagnosticiranega primera – Genotipski(HGVS)/Fenotipski(HPO) 18. Starost ob diagnozi v letih (Prenatalno/Ob rojstvu/Datum/Neznano) 19. Starost ob nastopu simptomov /znakov (Prenatalno/Ob rojstvu/ Datum/ Neznano) 20. Dodatne diagnoze (komodbidnosti) s šifro po MKB10 21. Klasifikacija funkcioniranja/oviranosti bolnika (Profil oviranosti/Točkovnik - WHO) iv. **Genetski podatki in biološki material**

	22. Za vsako od ugotovljenih različ ic: Ime gena z ugotovljeno različ ico (po HGNC nomenklaturi) ali kromosoma /HGVS klasifikacija/OMIM koda 23. Biološki material shranjen (Da/Ne) V. **Terapevtski sklop** 24. Podatek o zdravilu sirota glede na iskalnik EMA **Skupina neobveznih podatkov:** Antropometrič ni parametri in vitalni znaki (BMI, viš ina, teža, obseg glave, frekvenca dihanja, pulzi, temperatura) ; Preživetje/smrtnost ; Razvojni mejniki ; Kognitivne funkcije ; Kakovost življenja (QoL); Šolska izobrazba ; Smrtnost pre živetje; Neželen dogodek; Motorične zmogljivosti/izid ; Stopnja avtonomnosti ; Funkcionalnost organov (pljuč na, srčna, jetrna, oč i, itd.); Število nujnih sprejemov v bolnišnico; Stopnja dolgotrajnih komplikacij ; Pogostost okužb (ENT – uho, nos, grlo, pljuč a, itd.); Operacije
Enota opazovanja	Oseba
Kdo posreduje podatke v zbirko	V Register redkih bolezni RS podatke sprotno vnašajo bolnišnice RS (ambulantni in hospitalni oddelki, oddelki za citologijo/patologijo/genetiko) ter drugi zdravstveni zavodi na sekundarni in terciarni ravni, Centralni register prebivalcev RS, Register prostorskih enot RS. V zbirko Register redkih bolezni RS so na podlagi povezovalnega znaka EMŠO vsaj enkrat letno (oz. skladno s predpisano metodologijo Registra redkih bolezni RS) posredovani še podatki iz: - Zbirke podatkov o umrlih osebah (vzrok smrti, mesto smrti, številka mrliškega lista, bolnica, zdravnik in podatki o obdukciji za osebe z redko boleznijo kot osnovnim vzrokom smrti; za vse umrle, ki so registrirani v Registru redkih bolezni RS, pa vzrok smrti); - Zbirka podatkov o zunajbolnišničnih obravnavah pacientov na sekundarni in terciarni ravni zdravstvenega varstva (podatki za bolnike, ki so zboleli za redko boleznijo); - Zbirke podatkov o bolnišničnih obravnavah posameznikov v bolnišnicah in drugih stacionarnih ustanovah (s sprejemom v posteljno enoto) (podatki za bolnike, ki so zboleli za redko boleznijo); - Zbirk podatkov s področja zdravja in varstva pri delu (podatki za bolnike, ki so zboleli za redko boleznijo kot poklicno boleznijo); - Zdravstvene kartoteke (podatki za bolnike, ki so zboleli za redko boleznijo) in elektronske zdravstvene kartoteke . - Pacienti z redko boleznijo/svojci/skrbniki
Roki posredovanja podatkov v zbirko	Tekoče oz. skladno s predpisano metodologijo Registra redkih bolezni RS.
Rok hrambe podatkov v zbirki	Podatki se hranijo trajno.
Upravljavec zbirke	Pediatrična klinika , UKC Ljubljana , v sodelovanju z NIJZ. .
Upravičenci do podatkov iz zbirke	Osebne podatke lahko pridobijo samo zdravniki, ki so posredovali podatke, anonimizirane podatke lahko dobijo raziskovalci,ki imajo predhodno soglasje državne Komisije za medicinsko etiko in pacienti (svojci/skrbniki) , katerih podatki so v zbirki . Predvidena je izmenjava podatkov v sklopu Evropske platforme redkih bolezni (EU RD Platform).
Poročila	Upravljavec analizira podatke, pripravlja in objavlja obdobna poročila najmanj enkrat letno ter jih objavlja na svoji spletni strani.

NACIONALNI REGISTER DRU ŽINSKE HIPERHOLESTEROLEMIJE IN REDKIH DISLIPIDEMIJ - VPRA ŠALNIK
NATIONAL REGISTRY OF FAMILIAL HYPERCHOLESTEROLEMIA AND RARE DYSLIPIDEMIAS - QUESTIONNAIRE

Date of entry: _____
Date of patient's informed consent/authorization: _____
Person responsible for entry: _____

1. Patient and demographic data , identifiers

- First name:
- Last name:
- DOB:
- Place of birth:
- EMŠO:
- KZZ:
- Gender: M/F
- Ethnicity:
- Occupation:
- Education level:
- Phone/Email:
- Date of death:
- Cause of death:

2. Familial hypercholesterolemia score

- Clinical criteria used for diagnosis: DLCN/MedPed/Simon-Broome/other
- Clinical criteria score:
- Clinical criteria diagnosis: no diagnosis/possible/probable/confirmed

3. Familial hypercholesterolemia – diagnosis and genetic data

- Date of diagnosis:
- FH type: homozygous/heterozygous /compound heterozygous/ARH
- Consanguinity: yes/no
- Index case: yes/no
- Availability of family tree: yes/no
- Genetic study: positive/negative/not done
- FH defect: heterozygous/compound heterozygous/homozygous/ARH
- Mutation gene: LDLR/ApoB/PCSK9/LDLRAP1/Unknown
- Mutation type: stop/insertion/deletion/other
- Mutation result: defective allele/null allele
- Residual LDLR activity: ___%/unknown
- Variant id:
- Variant name:
- Date of report:
- Number of report:

4. Family history

- Family history of FH: yes/no
- Family history of hypercholesterolaemia: yes/no
- Family history of CAD: yes/no
- Family history of other CVD (stroke, PAD): yes/no
- 1st degree relative premature CAD: yes/no [premature: male <55y, woman <60y]

5. Clinical history and events

- Hypertension: yes/no

- Diabetes: type 1/type 2/other/no
- Hypertriglyceridemia: yes/no
- CAD: yes/no
- Premature CAD: yes/no [premature: male <55y, woman <60y]
- MI: yes/no
- Acute coronary syndrome: yes/no
- Coronary revascularization: yes/no
- Cerebral vascular disease: yes/no
- Stroke: yes/no
- Transient ischemic attack; yes/no
- Peripheral artery disease: yes/no
- Aortic valvular/supravalvular disease: yes/no
- Heart failure
- Premature non-coronary vascular disease: yes/no [premature: male <55y, woman <60y]
- CKD: yes/no
- CKD stage (KDOQI):
- Hepatic steatosis (suggested by US): yes/no
- Achilles tendon lesions: tendinitis/injury-surgery/no
- Cancer disease (type):
- Statin intolerance: clinical/biochemical/both

- Date of diagnosis/event

Social history

- Smoker: current/ quitting/former/never
- Pack-years smoking (number of cigarettes smoked per day divided by 20 and then multiplied by the number of years smoked): _____
- Alcohol: non-drinker/current drinker/former drinker
- Physical activity: yes/no
- Physical activity: _____min/wk

6. Examination

- Weight: ____ kg
- Height: _____ cm
- BMI: _____ kg/m^2
- Waist circumference: _____ cm
- Corneal arcus: yes/no
- Xanthomas: yes/no
- Xanthelasma: yes/no
- Systolic BP: _____ mmHg
- Diastolic BP: _____ mmHg
- Heart rate: _____ bpm

7. Laboratory profile

Highest levels recorded

- Total Cholesterol (mmol/L):
- LDL-C (m mol/L):

Pre-treatment lab

- Total Cholesterol (mmol/L):
- LDL-C (mmol/L):
- HDL-C (mmol/L):
- Non-HDL-C (mmol/L):
- Triglycerides (mmol/L):

- Apolipoprotein A1 (g/L):
- Apolipoprotein B (g/L):
- Lipoprotein (a) (mg/L):
- Creatin kinase (CK) (U/L):
- Aspartate aminotransferase (AST) (U/L):
- Alanine aminotransferase (ALT) (U/L):
- C-reactive protein (CRP) (mg/L):
- Glucose (mmol/L):
- Glycated haemoglobin (HbA1c) (%):
- Estimated glomerular filtration rate (eGFR): MDRD (ml/kg/m^2):
- Microlabuminuria (mg/g):

Most recent lab

- Total Cholesterol (mmol/L):
- LDL-C (m mol/L):
- HDL-C (m mol/L):
- Non-HDL-C (m mol/L):
- Triglycerides (m mol/L):
- Apolipoprotein A1 (g/L):
- Apolipoprotein B (g/L):
- Lipoprotein (a) (mg/L):
- Creatin kinase (CK) (U/L):
- Aspartate aminotransferase (AST) (U/L):
- Alanine aminotransferase (ALT) (U/L):
- C-reactive protein (CRP) (mg/L):
- Glucose (mmol/L):
- Glycated haemoglobin (HbA1c) (%):
- Estimated glomerular filtration rate (eGFR): MDRD (ml/kg/m^2):
- Microlabuminuria (mg/g):

8. Diagnostic exams

- ECG:
  - Rhythm: sinus rhythm/atrial fib/flutter/other
  - LVH (Sokolow-Lyon):
  - Other disorder:
- Echocardiography:
  - LVEF: ____ %
  - LV hyperthrophy: yes/no
  - Septum: _____ mm
  - Posterior wall: _____ mm
  - LV mass index: _____ g/m^2
  - Abnormal wall motion: yes/no
- Carotid Ultrasonography:
  - Carotid plaque: yes/no
  - Carotid (CCA) IMT: yes/no
  - Carotid atherosclerotic stenosis: _____ %/no
- Liver Ultrasonography: Liver steatosis: yes/no
- Renal arteries Ultrasonography: Stenosis: yes/no
- Lower extremity Ultrasonography: Stenosis: iliac/femoral/distal/multiple/no
- Ankle-brachial index: _____
- Coronary calcium score:
  - Method:
  - Score:
- Coronary angiography/revascularization:

- Coronary angiography: no lesions/diffuse disease/focal stenosis
- If stenosis: <50%/≥50%/>70%
- Coronary arteries affected: none/LCA/LAD/LCx/RCA/several
- PCI: yes/no
- CAGB: yes/no

Other test available:

9. Medications

Lipid-lowering medications

- Lipid-lowering therapy: no/monotherapy/combination therapy
- Statin: yes/no
- Type of statin: simvastatin/pravastatin/lovastatin/ fluvast atin/atorvastatin/rosuvastatin/pitavastatin
- Date starting statin (month/year):
- Dose of statin (mg/d):
- Statin intolerance: yes/no
- PCSK9 inhibitor: yes/no
- Existing indication for PCSK9: yes/no
- Type of indication for PCSK9: LDL-goal not achieved max. doses of therapy/statin intolerance/other
- Type of PCSK9: evolocumab/alirocumab
- Date starting PCSK9 (month/year):
- Dose of PCSK9 (mg/2w):
- Ezetimibe: yes/no
- Fibrate: yes/no
- Bile acid sequestrants: yes/no
- N-3 fatty acids: yes/no
- Niacin: yes/no
- Sterols/stanols: yes/no
- Lipoprotein apheresis: yes/no
- Lomitapide: yes/no
- Mipomersen: yes/no
- CEPT inhibitor: yes/no

Other medications

- Antiplatelet: yes/no
- ACEI: yes/no
- ARB: yes/no
- B-blocker: yes/no
- CCB: yes/no
- Other BP-lowering drug: yes/no
- Oral glucose-lowering drug: yes/no
- Insulin: yes/no

Adverse events

- Date of event:
- Description of event:
- Type of therapy:
- Dose of therapy
- Date of last application of therapy:
- This medication was the cause: yes/no
- Change of this medication was the cause: yes/no
- Other medication was the cause: yes/no
- Patient's improvement: spontaneous/after th change/ no

Goals of therapy and adherence

- Target value of LDL-C (mmol/L):
- Is the LDL-C goal achieved: yes/no
- The ratio between last LDL-C and target LDL-C (%):
- The total number of lipid-lowering medication doses missed during last month : name of therapy: _____ __; number of missed doses:_____ ; date:_____

Priloga 5 - Pedlagan nabor bolezni za vključitev v presejanje novorojencev za VBP z metodo MS/MS v Sloveniji:

- **fenilketonurija (PKU),**
- **bolezen javorjevega sirupa (MSUD),**
- **tirozinemija tip 1 (TYR1) [1],**
- **izovalerična acidemija (IVA),**
- **glutarična acidemija tip I (GAI),**
- **glutarična acidemija tip II (GAII),**
- **pomanjkanje zelo dolgoverižne acil-CoA dehidrogenaze (VLCAD),**
- **pomanjkanje dolgoverižne 3OH-CoA dehidrogenaze (LCHAD),**
- **pomanjkanje srednjeverižne acil-CoA dehidrogenaze (MCAD),**
- **propionska acidemia (PA),**
- **metilmalonska acidemija (MMA),**
- **pomanjkanje karnitine palmitoiltransferaze I (CPTI),**
- **pomanjkanje karnitine palmitoiltransferaze II (CPTII),**
- **motnja vnosa/transporta karnitina (CUD),**
- **pomanjkanje 3-metilkrotonil-CoA karboksilaze (3-MCC),**
- **3-hidroksi-3-metilglutari čna acidurija (HMG),**
- **pomanjkanje holokarboksilaze sintaze (MCD),**
- **pomanjkanje β-ketotiolaze (βKT).**

[1] V primeru tirozinemije bomo presejali v prvi fazi preko tirozina in metionina, ne bomo merili sukcinilacetona.

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Next generation sequencing as a follow-up test in an expanded newborn screening programme

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Next generation sequencing
Inborn errors of metabolism
Incidence

ABSTRACT

Objectives: Contrary to many western European countries, most south-eastern European countries do not have an expanded newborn screening (NBS) program using tandem mass spectrometry. This study would represent one of the first expanded NBS studies in south-eastern Europe and will enable the estimation of the incidences of IEM in Slovenia. We proposed an expanded NBS approach including next-generation sequencing (NGS) as a confirmational analysis.

Design & methods: We conducted a pilot study of expanded NBS for selected inborn errors of metabolism (IEM) in Slovenia including 10,048 NBS cards. We used an approach including tandem mass spectrometry followed by second tier tests including NGS. Based on the NBS results, 85 children were evaluated at a metabolic follow-up; 80 of them were analyzed using NGS.

Results: Altogether, glutaric acidemia type 1 was confirmed in one patient who was a compound heterozygote for two known causative *GCDH* variants. A patient with suspected very long-chain acyl-CoA dehydrogenase deficiency had negative metabolic follow-up tests, but had two heterozygous *ACADVL* variants; one known disease-causing variant and one indel, namely c.205-8_205-7delinsGC, that is predicted to be causative. Nine participants had elevated metabolites characteristic of 3-methylcrotonyl-CoA carboxylase deficiency, 2 of them had known causative homozygous variants in *MCCC1*. The other seven were heterozygous; two had a novel genetic variant c.149_151dupCCA (p.Thr50dup). Cumulative incidences of IEM in Slovenia were similar to other European countries.

Conclusions: NGS proved to be valuable in explaining the abnormal metabolite concentrations in NBS as it enabled the differentiation between affected patients and mere heterozygotes, and it improved the turnaround time of genetic analysis. The results of this study will be instrumental in the routine implementation of expanded NBS in Slovenia.

1. Introduction

Inborn errors of metabolism (IEM) can pose a major health problem with consequences ranging from minor disabilities to sudden death [1]. The development of various screening tests to identify IEM in newborns had a major impact on the outcome of the affected patients as, for many of the disorders, clinical signs can be prevented with proper treatment, which in most cases involves a special dietary program [2]. Beginning with a bacterial inhibition test for phenylketonuria (PKU) [3,4], tests for additional IEMs were developed, thus enabling an expansion of newborn screening (NBS) programs [2]. Nowadays, tandem mass spectrometry (MS/MS) allows screening for several amino acid metabolism disorders, fatty acid oxidation disorders and organic acidemias in one brief but sensitive analysis [5]. Most European countries screen newborns for congenital hypothyroidism and PKU [6,7] and many of them have an expanded NBS program using MS/MS and screen for > 10 IEMs [6]. However, there is no expanded NBS program in any south-eastern European country [4,7,8] and some of the countries in the region have no NBS program at all [4,7,8].

Next generation sequencing (NGS) is developing rapidly and is offering the simultaneous analysis of numerous genes accounting for numerous disorders. Therefore, it is becoming a method of choice for newborn screening. There is strong interest in implementing NGS into NBS programs [9], but there are still limited reports on its implementation. Only one study demonstrates using NGS as a first-tier test in newborn screening [10] and more in genetic diagnostics when there

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is a clinical suspicion of an IEM [11,12]. In one, NGS was a second tier confirmational test [13]. Implementing NGS as a first tier test could allow the expansion of screened disorders beyond just IEMs. NGS as a follow-up test could allow a faster final confirmation of the disease in cases when enzyme activity analysis or the detection of causative genetic variants is necessary for confirmation. In the light of the possibilities that NGS is offering, we proposed an expanded NBS approach including NGS as a confirmational analysis. This study would represent one of the first expanded NBS studies in south-eastern Europe and would enable the estimation of incidences of IEM in Slovenia. We assumed that NGS is a possible method for a follow-up test after an abnormal NBS result and compared its effectiveness in our cohort with the recommended follow-up tests: analysis of organic acids in urine, amino acids in plasma and an additional acylcarnitine test using dried blood spots (DBS).

2. Materials and methods

2.1. Subjects

10,048 Slovenian NBS cards from neonates born in 2013 and 2014 were included in the study. DBSs were taken from 48 h to 72 h after birth for ongoing NBS for PKU and congenital hypothyroidism. Samples were analyzed retrospectively, the average age of the samples at the time of analysis was 8.5 months (from 6 to 11 months). 113 children with the highest probability for each of the tested IEMs (Table 1) were invited for an outpatient visit at the University Children's Hospital and further confirmation of the NBS results. All those unresponsive to our first invitation were invited again. Finally, 85 participants (75%) responded to our invitation and their parents were informed about the study and asked for a written informed consent for further participation in the study. We collected all the required samples (DBSs, urine, whole blood with EDTA, whole blood with lithium heparin) for 73 participants (86%), we were unable to acquire DNA samples from 5 of them (6%) and urine samples from 7 (8%). Follow-up analyses were based on algorithms formed by the American College of Medical Genetics and Genomics [14]. Parents of the patients with very long-chain acyl-CoA dehydrogenase deficiency (VLCAD) and with glutaric acidemia type 1 (GA 1) were tested for the presence of family variants detected in the probands. The principles of the Declaration of Helsinki were followed and the study was approved by the Slovenian national Medical Ethics Committee (#56/01/14).

2.2. Methods

2.2.1. Dried blood spot analysis of acylcarnitines and amino acids

Blood spot samples were analyzed using the PerkinElmer 200 HPLC system coupled to AB Sciex 3200 QTRAP (AB Sciex, Singapore) using Chromsystems' kit Amino Acids and Acylcarnitines from Dried Blood (Grüfelfing, Germany). A 3 mm disk was punched from the DBS, analytes were extracted using the extraction buffer with added internal standards. Analytes were derivatized to butyric esters, the derivatization reagent was evaporated and the samples were analyzed after reconstitution in the reconstitution buffer.

The results of the analysis and analytes ratios associated with the IEMs included in our study are listed in Table 1.

2.2.2. Urine organic acids analysis

Urine organic acids were measured using an in-house method described previously [15]. To 1 mL of urine in a glass tube, 20–30 mg of O-ethylhydroxylamine (Aldrich, Switzerland) was added and the solution was incubated for 15 min at room temperature. Then we first added 100 mmol of 2-phenylbutyric acid (Aldrich, Ireland) per mol of creatinine, followed by 100 µL of 4 N HCl (prepared from hydrochloric acid ≥ 37%, Sigma-Aldrich, Germany). Afterwards, the solution was saturated with NaCl (Sigma-Aldrich, Denmark) and 2 mL of ethyl acetate (Sigma-Aldrich, Mexico). The solution was subsequently vortexed and centrifuged for 5 min at 1850*g*. 1 mL of supernatant was transferred to a clean glass tube and the ethyl acetate was evaporated under a stream of nitrogen. Finally, 50 µL of pyridine (Sigma-Aldrich, India) and 200 µL of *N*,*O*-bis(trimethylsilyl)trifluoroacetamine (Aldrich, USA) were added, followed by analysis using the Agilent 5975C Series GC/MSD (Agilent Technologies, USA) on an Agilent Ultra2 column 30 m × 0.2 mm × 0.33 µm (Agilent Technologies, USA).

2.2.3. Blood plasma amino acid analysis

Amino acids were quantified using the same LC-MS/MS system as acylcarnitine analysis, with an aTRAQ™ Kit for Physiological Fluids (SCIEX, USA). In the first step, proteins were precipitated with sulphosalicylic acid. Amino acids were labeled with aTRAQ reagent according to the manufacturer's instructions. For quantification, the internal standards of each analyzed amino acid were used. Amino acids were separated on an HPLC system with a C18 column (part of aTRAQ kit) and analyzed on a triple quadrupole in MRM mode.

Table 1
Measured analytes and their ratios, as well as IEM associated genes included in the NGS.

IEM	Measured analytes and their ratios	Analyzed genes
MCAD	C6, C8, C10, C10:1, C8/C10, C8/C2	*ACADM* (NG_007045.2)
GA 1	C5DC, C5DC/C5OH, C5DC/C8, C5DC/C16	*GCDH* (NG_009292.1)
3-MCC[a]	C5OH, C5OH/C8	*MCCC1* (NG_000100.1), *MCCC2* (NG_008882.1), *HMGCL* (NG_000191.2), *ACAT1* (NG_009888.1), *HSD17B10* (NG_008153.1), *AUH* (NG_008017.,1), *BTD* (NG_008019.1), *HLCS* (NG_016193.1)
MSUD	Xle, Val, Xle/Phe, Xle/Ala, Val/Phe	*BCKDHA* (NG_013004), *BCKDHB* (NG_009775), *DBT* (NG_011852), *DLD* (NG_008045)
VLCAD	C14, C14:1, C14:1/C2, C14:1/C16	*ACADVL* (NG_007975.1)
IVA	C5, C5/C0, C5/C2, C5/C3	*IVD* (NG_011986.1), *ACADSB* (NG_008003.1)
GA 2	C4, C5, C8, C5DC, C14, C16, C14:1/C2, C8/C2, C5/C2, C5/C3, C4/C3, C4/C2, C5DC/C5OH, C14:1/C16	*ETFA* (NG_007077.2), *ETFB* (NG_007115.1), *ETFDH* (NG_007078.2), *ETHE1* (NG_008141.1)
LCHAD	C16:1OH, C16OH, C18:1OH, C18OH, C16 OH/C16	*HADHA* (NG_007121.1), *HADHB* (NG_007294.1)
MMA & PA[a]	C3, C3/C2, C3/C16	*SUCLA2* (NG_008241.1), *MUT* (NG_007100.1), *MMAA* (NG_007536.1), *MMAB* (NG_007096.1), *MMACHC* (NG_013378.1), *MMADHC* (NG_009189.1), *LMBD1* (NG_016012.1), *TCN2* (NG_007263.1), *PCCA* (NG_008768.1), *PCCB* (NG_008939.1), *SUCLG1* (NG_016755.1)
CUD	C0, AC/Cit	*SLC22A5* (NG_008982.1)
CPT 1	C0, C16, C18, C0/(C16 + C18)	*CPT1A* (NG_011801.1)
CPT 2	C14, C16, C18, (C16 + C18:1)/C2	*CPT2* (NG_008035.1), *SLC25A20* (NG_008171.1)

AC: acetylcarnitine (C2) + propionylcarnitine (C3) + palmitoylcarnitine (C16) + stearoylcarnitine (C18) + oleylcarnitine (C18:1); 3-MCC: 3-methylcrotonyl carboxylase deficiency; CPT 1/2: carnitine palmitoyltransferase deficiency type 1/2; CUD: carnitine uptake deficiency; GA 1/2: glutaric acidemia type 1/2; IVA: isovaleric acidemia; LCHAD: long-chain acyl CoA dehydrogenase deficiency; MMA: methylmalonic acidemia; MSUD: maple syrup urine disease; PA: propionic acidemia; Xle: Leu + Ile.

[a] Some of the genes analyzed are associated with IEM not included in the study, but have the same elevations of metabolites in dried blood spots as the included IEM.

2.2.4. Enzyme measurements

Measurement of enzyme activities in fibroblasts and lymphocytes and acylcarnitine profiling (palmitate loading test) were performed in the Academisch Medisch Centrum, Laboratorium Genetische Metabole Ziekten, Amsterdam, Netherlands, as part of the offered commercial testing.

2.2.5. Genetic analysis

DNA was isolated from whole blood using established laboratory protocols based on the FlexiGene DNA isolation kit (Qiagen, Hilden, Germany). Sample preparation for NGS proceeded according to the protocols of the manufacturers of the preparation kits used. The first step was fragmentation with NEBNext dsDNA fragmentase (New England Biolabs, UK). A DNA library was prepared using the NEBNext DNA Library Prep Master Mix Set for Illumina (New England Biolabs, UK). Samples were purified using sample purification beads (Illumina) and indexed with NEBNext multiplex oligos for Illumina (New England Biolabs, UK). The library enrichment with hybridization probes was done using the IDT Hybridization Capture of DNA Libraries kit with xGen Lockdown Probes and Reagents (Integrated DNA Technologies, USA), enabling the sequencing of exon regions extending 10 nucleotides into the intronic regions of selected genes. Samples were multiplexed in two groups of 47 and 33 samples and were sequenced in two runs. The samples were sequenced using an Illumina MiSeq with MiSeq Reagent kit v3 (600 Cycle) (Illumina, USA). The post-sequencing analysis (alignment and variant calling) and ROI coverage analysis were performed using a NextGENe 2.4.1.2. (SoftGenetics, USA) followed by variant filtering and annotation performed with the Illumina Variant Studio 2.2 using the GRCh37.p13 assembly of the human genome as a reference sequence and the variant annotation database provided by the Illumina Variant Studio software. Regions with $< 10\times$ coverage and genetic variant with MAF > 1% in the general population according to the ExAc Browser (http://exac.broadinstitute.org/) were excluded from the analysis.

All patients with possible causative genetic variants identified by NGS testing were re-analyzed using Sanger sequencing to confirm the presence of the variant. For participants with a prominent risk of false negative follow-up results: long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency (LCHAD), VLCAD, GA 1, carnitine palmitoyltransferase deficiency type 2 (CPT 2) [14], and participants with measured elevated metabolites for 3-methylcrotonyl-CoA carboxylase deficiency (3-MCC) at follow-up, additional Sanger sequencing of poorly covered exons was performed. Regions were Sanger sequenced using primers designed in-house (sequences available upon request), a BigDye Terminator v3.1 sequencing kit and an ABI Genetic Analyzer 3500 (both Applied Biosystems, Foster City, USA). Two participants, one with a classical PKU and one with hyperphenylalaninemia, were already confirmed within the existing screening program with Sanger sequencing.

We performed *in situ* prediction analyses for all novel variants previously not reported in patients with IEM or in the general population. For point mutations, SIFT (http://sift.jcvi.org/), PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/) and Mutation Taster (http://www.mutationtaster.org/) prediction tools were used. For the novel intronic variant and duplication, Mutation Taster and CADD (http://cadd.gs.washington.edu/) were used. The prediction anticipated by at least two tools prevailed. The guidelines for diagnostic next-generation sequencing including the methodology and variant interpretation issued by the European Society for Human Genetics were followed [16].

2.2.6. Estimation of the incidences of IEMs

An estimation of the combined incidences of all IEMs tested in this study was performed using the calculated incidence from the tested cohort of 10,048 newborns and the number of clinically confirmed cases in Slovenia born between 1999 and 2013 (the total number of newborns born in these years was acquired from the Statistical Office of the Republic of Slovenia [17]). The incidence of PKU in Slovenia used for the calculation of cumulative incidences of IEMs in Slovenia was 1:6769 [8]. The estimated incidence for all the tested IEMs was calculated as an average of NBS incidence and incidence in clinically detected cases. For each IEM that was detected in both NBS and clinical evaluation, the incidence was calculated as an average of the incidences. The estimated incidence of 3-MCC and VLCAD, which were only detected in this pilot study and had never been previously detected in Slovenia, are therefore the same as their incidences in the pilot study. The cumulative estimated incidences of glutaric acidemia type 2 (GA 2), LCHAD, methylmalonic acidemia (MMA) and propionic acidemia (PA), which were not identified in this pilot study, are the same as the incidences in previous clinically detected cases.

3. Results

3.1. DBS screening results

Results for each of the measured acylcarnitines, amino acids and their ratios (Table 1) were categorized into highest and lowest and 113 children with the highest or lowest results were selected for follow-up testing (Table 2). The cut-offs given in Table 1 were set after the completion of the study at the 99.9th percentile of the measured concentrations of each analyte. Among them, the share of preterm infants (gestation age < 37 weeks) was 50–70% in maple syrup urine disease (MSUD), isovaleric acidemia (IVA), GA 2 and carnitine palmitoyl transferase deficiency type 1 (CPT 1), 20–40% in PA, MMA, GA 1, medium-chain acyl-CoA dehydrogenase deficiency (MCAD), VLCAD and carnitine uptake deficiency (CUD), while in CPT 2, 3-MCC and LCHAD the share was 10% or less. In total, 33% of the newborns we followed-up were preterm infants.

Table 2
The break down by IEM of the 113 first screen positive samples. In the brackets the number of participants, who were positive after newborn screening for more than one IEM, is written.

IEM	No follow-up (overlap)	Responded (overlap)	Organic acids (overlap)	Amino acids	Acylcarnitines and amino acids in DBS (overlap)	NGS (overlap)
3-MCC	14 (0)	11 (0)	9 (0)	x	11 (0)	11 (0)
VLCAD	11 (4)	10 (4)	x	x	10 (4)	10 (4)
GA 1	11 (4)	9 (4)	9 (4)	x	9 (4)	9 (4)
MCAD	10 (4)	9 (4)	9 (4)	x	9 (4)	9 (4)
GA 2	11 (9)	10 (9)	9 (8)	x	9 (8)	10 (9)
LCHAD	11 (1)	8 (1)	6 (1)	x	8 (1)	6 (1)
MMA & PA	13 (2)	8 (2)	8 (2)	x	8 (2)	7 (2)
CPT 1	10 (1)	6 (1)	x	x	6 (1)	6 (1)
CPT 2	10 (1)	7 (1)	x	x	7 (1)	6 (1)
CUD	11 (2)	10 (2)	x	x	10 (2)	10 (2)
IVA	10 (2)	9 (2)	8 (1)	x	9 (2)	9 (2)
MSUD	10 (2)	7 (2)	6 (1)	7 (1)	x	6 (1)

Table 3
Participants of the study with heterozygotic or homozygotic genetic variants that are associated with elevations in their newborn DBS. Confirmed IEM and novel genetic variants named at the DNA and protein level are written in bold. P – participant.

P	IEM	NBS result	Follow-up	Genetic variant
1	**GA 1**	C5DC = 5.5 μmol/L(< 0.25 μmol/L)	C5DC = 2.1 μmol/L (< 0.15 μmol/L) C0 = 4.3 μmol/L (15.5–46.7 μmol/L) Elevated glutaric and 3-hydroxyglutaric acid	*GCDH* NM_000159.3:c.[1060G > A];[1204C > T] NP_000150.1:p.[Gly354Ser];[Arg402Trp]
2	**VLCAD**	C14:1 = 0.52 μmol/L(< 0.19 μmol/L)C14 = 0.49 μmol/L(< 0.40 μmol/L)C14:1/C2 = 0.026(< 0.011)C14:1/C16 = 0.17 (< 0.08)	Normal	*ACADVL* NM_000018.3:c. [1837C > T(;)205-8_205-7delinsGC] NP_000009.1: p.[Arg613Trp(;)?]
3	VLCAD	C14:1 = 0.22 μmol/L (< 0.19 μmol/L)C14 = 0.30 μmol/L(< 0.40 μmol/L)C14:1/C2 = 0.012(< 0.011)C14:1/C16 = 0.08 (< 0.08)	Normal	*ACADVL* NM_000018.3:c.[1837C > T];[=] NP_000009.1:p.[Arg613Trp];[=]
4	VLCAD	C14:1 = 0.21 μmol/L (< 0.19 μmol/L)C14 = 0.25 μmol/L(< 0.40 μmol/L)C14:1/C2 = 0.009(< 0.011)C14:1/C16 = 0.17 (< 0.08)	Normal	*ACADVL* NM_000018.3:c.[1076C > T];[=] NP_000009.1:p.[Ala359Val]:[=]
5	VLCAD	C14:1 = 0.25 μmol/L (< 0.19 μmol/L)C14 = 0.28 μmol/L(< 0.40 μmol/L)C14:1/C2 = 0.012(< 0.011)C14:1/C16 = 0.07 (< 0.08)	Normal	*ACADVL* NM_000018.3:c.[1843C > T];[=] NP_000009.1:p.[Arg615Ter]:[=]
6	**3MCC**	C5OH = 7.72 μmol/L (< 0.56 μmol/L)C5OH/C8 = 275.7 (< 16.4)	C5OH = 18.82 μmol/L (< 0.43 μmol/L) C5OH/C8 = 869.3 (< 10.0) Elevated 3-hydroxyisovaleric acid and 3-methylcrotonylglycine	*MCCC1* NM_020166.4:c.[558delA];[558delA] NP_064551.3:p.[Gln186HisfsTer6];[Gln186HisfsTer6]
7	**3MCC**	C5OH = 4.66 μmol/L (< 0.56 μmol/L)C5OH/C8 = 16.9 (< 16.4)	DBS analysis not done Elevated 3-hydroxyisovaleric acid and 3-methylcrotonylglycine	*MCCC1* NM_020166.4:c.[1155A > C];[1155A > C] NP_064551.3:p.[Arg385Ser];[Arg385Ser]
8	3MCC	C5OH = 0.56 μmol/L (< 0.56 μmol/L) C5OH/C8 = 2.23 (< 16.4)	C5OH = 1.13 μmol/L (< 0.43 μmol/L) C5OH/C8 = 34.2 (< 10.0) Elevated 3-hydroxyisovaleric acid and 3-methylcrotonylglycine	*MCCC1* NM_020166.4:c.[558delA];[=] NP_064551.3:p.[Gln186HisfsTer6];[=]
9	3MCC	C5OH = 0.46 μmol/L (< 0.56 μmol/L) C5OH/C8 = 16.2 (< 16.4)	Normal	*MCCC1* NM_020166.4:c.[1155A > C];[=] NP_064551.3:p.[Arg385Ser];[=]
10	3MCC	C5OH = 0.53 μmol/L (< 0.56 μmol/L) C5OH/C8 = 19.1 (< 16.4)	C5OH = 0.76 μmol/L (< 0.43 μmol/L) C5OH/C8 = 10.3 (< 10.0) No urine sample	*MCCC1* NM_020166.4:c.[1155A > C];[=] NP_064551.3:p.[Arg385Ser]:[=]
11	3MCC	C5OH = 0.76 μmol/L (< 0.56 μmol/L) C5OH/C8 = 10.2 (< 16.4)	C5OH = 0.58 μmol/L (< 0.43 μmol/L) C5OH/C8 = 9.3 (< 10.0) No urine sample	*MCCC1* NM_020166.4:c.[1155A > C];[=] NP_064551.3:p.[Arg385Ser];[=]
12	3MCC	C5OH = 0.70 μmol/L (< 0.56 μmol/L) C5OH/C8 = 21.84 (< 16.4)	Normal	*MCCC1* NM_020166.4:c.[2079delA];[=] NP_064551.3:p.[Val694Ter];[=]
13	3MCC	C5OH = 0.742 μmol/L (< 0.56 μmol/L) C5OH/C8 = 8.73 (< 16.4)	C5OH = 0.46 μmol/L (< 0.43 μmol/L) C5OH/C8 = 5.4 (< 10.0) No urine sample	*MCCC1* NM_020166.4:**c.[149_151dupCCA]**;[=] NP_064551.3:**p.[Thr50dup]**;[=]
14	3MCC	C5OH = 0.70 μmol/L (< 0.56 μmol/L) C5OH/C8 = 16.2 (< 16.4)	C5OH = 0.644 μmol/L (< 0.43 μmol/L) C5OH/C8 = 71.6 (< 10.0) Elevated 3-methylcrotonyl glycine	*MCCC1* NM_020166.4:**c.[149_151dupCCA]**;[=] NP_064551.3:**p.[Thr50dup]**;[=]
15	CUD	C0 = 6.3 (7.7–64 μmol/L) AC/Cit = 1.52 (> 0.79)	C0 = 10.6 (15.5–46.7 μmol/L) AC/Cit = 1.2 (> 1.9 μmol/L)	*SLC22A5* NM_003060.3:c.[136C > T];[=] NP_003051.1: p.[Pro46Ser];[=]
16	**cPKU**	Phe = 520 μmol/L (< 104 μmol/L) Phe/Tyr = 15.3 (< 1.9 μmol/L)	Not done	*PAH* NM_000277.1:c.[1222C > T(;)473G > A] NP_000268.1: p.[Arg408Trp(;)Arg158Gln]
17	HFA	Phe = 415 μmol/L (< 104 μmol/L Phe/Tyr = 6.3 (< 1.9 μmol/L)	Not done	*PAH* NM_000277.1:c.[678G > C];[=] NP_000268.1:p.[Gln226His];[=]

AC - acetylcarnitine (C2) + propionylcarnitine (C3) + palmitoylcarnitine (C16) + stearoylcarnitine (C18) + oleylcarnitine (C18:1); C0 – free carnitine; C5DC – glutarylcarnitine; C5OH – 3-hydroxy isovalerylcarnitine and 2-methyl 3-hydroxy butyrylcarnitine; C8 – octanoylcarnitine,Cit – citrulline; cPKU – classical phenylketonuria; HFA – hyperphenylalaninemia; Phe – phenylalanine; Tyr – tyrosine.

Table 4
Estimated incidences of IEM in Slovenia. For comparison average incidences of IEM in Europe are given, calculated from the available published data.

Disease	No. of cases in the pilot study of 10,048 newborns (incidence)	No. of clinically detected cases out of 293,897 newborns born from 1999 to 2013 (incidence)	Estimated incidence of IEM in Slovenia	European incidences [38–40]
All tested IEM	4 (1:2512)	9 (1:32,655)	1: 4665 (1: 2762 incl. PKU with incidence 1:6769)	1: 6000 (1: 3500 incl. PKU with incidence 1: 8500)
3-MCC	2 (1:5024)	0 (< 1:293,897)	1:5024	1: 80,000
VLCAD	1 (1:10,048)	0 (< 1:293,897)	1:10,048	1: 148,000
GA 1	1 (1:10,048)	3 (1:97,966)	1: 54,007	1: 76,500
MCAD	0 (< 1:10,048)	2 (1:146,949)	1: 146,949	1: 11,000
GA 2	0 (< 1:10,048)	1 (1:293,897)	1: 293,897	1: 28,100
LCHAD	0 (< 1:10,048)	1 (1:293,897)	1: 293,897	1: 146,000
MMA	0 (< 1:10,048)	1 (1:293,897)	1: 293,897	1: 177,000
PA	0 (< 1:10,048)	1 (1:293,897)	1: 293,897	1: 164,000
CPT 1	0 (< 1:10,048)	0 (< 1:293,897)	< 1: 293,897	1: 171,000
CPT 2	0 (< 1:10,048)	0 (< 1:293,897)	< 1: 293,897	1: 250,000
CUD	0 (< 1:10,048)	0 (< 1:293,897)	< 1: 293,897	1: 221,000
IVA	0 (< 1:10,048)	0 (< 1:293,897)	< 1: 293,897	1: 342,000
MSUD	0 (< 1:10,048)	0 (< 1:293,897)	< 1: 293,897	1: 281,000

3-MCC: 3-methylcrotonyl carboxylase deficiency; CPT 1/2: carnitine palmitoyl transferase deficiency type 1/2; CUD: carnitine uptake deficiency; GA 1/2: glutaric acidemia type 1/2; IVA: isovaleric acidemia; LCHAD: long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency; MMA: methylmalonic acidemia; MSUD: maple syrup urine disease; PA: propionic acidemia, PKU: phenylketonuria.

3.2. Follow-up tests

85 participants responded to our invitations. We identified 9 participants with disease-specific elevations of metabolites in the follow-up tests (Table 3). We included 73 participants in NGS-based genetic testing. The percent of ROI exceeding 10 × coverage for the whole tested gene panel was 84% (SD = 12%) and the average sequencing coverage was 267 ×.

One patient (participant 1 in Table 3) had elevated values of glutaric, 3-hydroxy glutaric and acetoacetic acid in urine. This patient had no detectable glutaryl-CoA dehydrogenase activity in the lymphocytes and was identified as a compound heterozygote for two known disease-causing variants in *GCDH*; c.1060G > A (p.Gly354Ser) [18] inherited paternally and c.1204C > T (p.Arg402Trp) [19] inherited maternally. At the age of one and a half years when the diagnosis was confirmed, the participant presented delayed development but no other clinical signs of GA 1.

In participant 2 (Table 3), with elevated metabolites characteristic of VLCAD, the repeated test of acylcarnitines in the DBS was within the normal ranges. Per ACMG recommendations [14], additional analyses were performed, namely enzyme activity analysis, acylcarnitine profiling in the lymphocytes and fibroblasts and *ACADVL* gene sequencing. NGS identified an already described disease-causing heterozygotic variant, c.1837C > T p.Arg613Trp [20], inherited paternally, and a known intronic variant c.205-8_205-7delinsGC, inherited maternally, predicted to be causative with Mutation Taster and CADD (Phred score 18.5). The participant had 68% of the normal enzymatic activity in the lymphocytes (1.26 nmol/(min mg protein)), reference values 1.84–4.80 nmol/(min mg protein)), and 20% of the normal enzymatic activity in the fibroblasts (0.30 nmol/(min mg protein)), reference values 1.48–5.34 nmol/(min mg protein)). Elevated levels of C12 acylcarnitine 3.0 nmol/(4 days × mg protein) (cut-off < 0.8 nmol/(4 days × mg protein)) and C14 acylcarnitine 1.8 nmol/(4 days × mg protein) (cut-off < 0.4 nmol/(4 days × mg protein)) were measured using acylcarnitine profiling (palmitate loading test). The results are conclusive of a mild form of VLCAD. Participants 3, 4 and 5 (Table 3) had minor elevations of acylcarnitines for VLCAD in the DBS and normal results in the follow-up tests; all were heterozygous for known disease-causing variants, namely c.1837C > T (p.Arg613Trp) [20], c.1076C > T (p.Ala359Val) [21] and c.1843C > T (p.Arg615Ter) [22] in *ACADVL* gene.

Nine participants with suspected 3-MCC had elevated 3-hydroxyisovaleric acid and 3-methylcrotonylglycine in urine and/or elevated C5OH and C5OH/C8 in DBS. 3-MCC in participants 6 and 7 (Table 3) was confirmed with a 3-MCC deficiency as they had abnormal metabolites in urine and DBSs, as well as the already reported homozygous disease-causing variants c.558delA (p.Gln186HisfsTer6) [23] and c.1155A > C (p.Arg385Ser) [24] in the *MCCC1* gene. In participants 9 and 12 (Table 3), who had elevations specific for 3-MCC only in their newborn DBSs and normal metabolic follow-up results, one known heterozygous disease-causing variant in each, namely c.1155A > C (p.Arg385Ser) [24] and c.2079delA (p.Val694Ter) [25] in the *MCCC1* gene was found. Participants 8, 10, 11, 13 and 14 (Table 3) had minor elevations of metabolites at follow-up and had heterozygous genetic variants in *MCCC1*: known disease-causing variant c.558delA (p.Gln186HisfsTer6) [23] in participant 8, known disease-causing variant c.1155A > C (p.Arg385Ser) [24] in participants 10 and 11 and a novel variant c.149_151dupCCA (p.Thr50dup) in participant 13 and 14 predicted to be benign with Mutation Taster and CADD (Phred score 9.0).

Four other heterozygous variants in the *MCCC1* and *MCCC2* genes were also identified in four participants who did not have elevations of 3-MCC specific metabolites at any time. These were disease-causing variants in *MCCC1* c.893T > A (p.Val298Glu), *MCCC1* c.1055G > A (p.Gly352Glu), *MCCC2* c.396G > A (p.Met132Ile), and a benign variant in *MCCC1* c.286T > C (p.Tyr96His).

Participant 15 with decreased free carnitine (C0) in the newborn DBS and a decreased C0 concentration and AC/Cit ratio (acetylcarnitine (C2) + propionylcarnitine (C3) + palmitoylcarnitine (C16) + stearoylcarnitine (C18) + oleylcarnitine (C18:1))/citrulline) at follow-up was found to be heterozygous for a known disease-causing variant c.136C > T (p.Pro46Ser) in *SLC22A5* gene [26].

3.3. Estimated incidences of IEMs in Slovenia

Incidences of each of the tested IEMs and the cumulative incidence of IEMs were calculated separately from the results of the pilot NBS study and clinically detected cases of IEMs. As the type and number of IEMs detected in the pilot study were different from the clinically detected IEMs, the final estimation of incidences of IEMs in Slovenia was calculated using both the results of the pilot study and the number of clinically detected cases. These estimations (Table 4) are the most accurate estimation of IEMs currently possible in Slovenia.

4. Discussion

The currently ongoing national NBS program in Slovenia only includes congenital hypothyroidism and PKU. Here we have conducted a

pilot expanded NBS study with an approach including NGS and consequently the expanded NBS program is being implemented in Slovenia in 2017.

Out of 10,048 newborn blood spot samples tested, 113 candidate participants were invited to follow-up analyses and 85 participants responded to the invitation. One of the reasons for the 25% dropout may be that some participants might have changed their home address and therefore did not receive the invitation letter. A few might also have ignored the invitation, despite receiving an additional invitation, as the candidate participant was apparently healthy. Twelve participants that responded to the invitation did not deliver all the required samples. 33% of the children tested at follow-up were born premature, which is a much larger proportion than the 7% of preterm infants born annually in Slovenia [27]. The changed levels of metabolites in premature children and therefore their increased rate among participants invited for the follow-up studies is both because of the physiologically different concentrations of metabolites [28] and because of parenteral nutrition, which can contain varying amounts of carnitine, amino acids and fatty acids [29]. Premature newborns can have false normal concentrations of metabolites in the DBSs, requiring separate monitoring and possibly an additional DBS test within one month of the birth [30].

There were nine participants (participants 6–14) with an elevated metabolite specific for 3-MCC. The NGS was informative as a final confirmation since it enabled the differentiation between true patients with 3-MCC and participants with elevated metabolites due to heterozygosity. Mothers of the participants were not tested for a possible maternal 3-MCC. In two participants, 3-MCC was confirmed with the detection of the known homozygous disease-causing *MCCC1* variant c.558delA (p.Gln186HisfsTer6) in participant 6 [23] and c.1155A > C (p.Arg385Ser) in participant 7 [24]. Participant 8 had the same genetic variant as participant 6 in a heterozygous form, as well as elevated acylcarnitines in the DBS and elevated organic acids. The same genetic variant as in participant 7 was detected in a heterozygous form in 3 other participants (participants 9, 10, 11), where one had all the analytes normal at follow-up and the other two had slightly elevated concentrations of C5OH (3-hydroxy isovalerylcarnitine and 2-methyl 3-hydroxybutyrylcarnitine) or the ratio C5OH/C8 (octanoylcarnitine) and one of them had normal organic acids in urine (organic acids analysis was not done in the third patient). This is in slight contrast with the previous reports [23,31], where all children with this heterozygous mutation had elevations of at least some 3-MCC-specific metabolites. The difference between the heterozygotes could be caused by different metabolic demands on the metabolic pathway, influenced by diet and environmental stress factors, or by a difference in allelic expression between the wild-type and affected allele [31]. Participant 12, with the known heterozygotic disease-causing *MCCC1* variant c.2079delA (p.Val694Ter) [25], also had normal metabolic follow-up results. The genetic variant c.149_151dupCCA (p.Thr50dup) predicted to be benign by *in silico* models and identified in participants 13 and 14 had not previously been described. In the heterozygous state, it led to an elevation of metabolites in the DBSs and elevated 3-methylcrotonylglycine in participant 14. Unfortunately, the urine sample of participant 13 was not available. In participants with elevations of C5OH and C5OH/C8 in their newborn DBS but not at follow-up, heterozygotic genetic variants were also detected. Heterozygotes, found in NBS for IEMs, are treated as false-positives because the diseases are autosomal recessive disorders and heterozygotes are normally not affected [32]. In some IEMs, the affected patients cannot be separated from heterozygotes and maternal IEMs based on NBS results alone [33], but for most, including 3-MCC, increasing the cut-off values enables this kind of separation [33]. Our cut-off values of C5OH and C5OH/C8 for 3-MCC, set at 99.9th percentile after the completion of the present study, enabled the separation of heterozygotes and homozygotes. Additionally, an international exchange of samples along with external quality control (*e.g.* ERNDIM, INSTAND, CDC) is valuable for accurate detection of very rare disorders.

The sequencing coverage in NGS was less than ideal with an average of 84% of ROI covered > 10 × times. The relatively sub-par performance of the prepared NGS libraries may be attributed to the non-optimized region of interest capture conditions, which would require additional optimization steps (to improve enrichment) and reagent selection (to improve the overall quality of the NGS library). With improved library preparation protocols, the multiplexing of the samples should not affect the quality of sequencing data. To further improve the coverage, we complemented the NGS technique with Sanger sequencing, with which we sequenced all the targeted regions with insufficient NGS coverage (< 10 × depth of coverage). After Sanger sequencing, all the genes associated with LCHAD, GA 1, GA 2 and VLCAD, as well as *MCCC1* and *MCCC2* genes in participants with elevated metabolites at follow-up, were fully covered. To implement NGS as a routine follow-up test, better sequencing coverage would be required, warranting a protocol where coverage of 95% should be an achievable goal [10]. Nevertheless, NGS proved to be beneficial in our cases since it helped explain the metabolite results, as well as improving the turnaround time for genetic analysis. The use of NGS as a second tier test for IEM confirmation after a positive NBS has so far been reported in only one study [13] and our study provides further proof of the utility of NGS in NBS. The results of the metabolic follow-up tests yielded 9 children with elevated specific metabolites with 5 of them being false positives, while NGS correctly identified only the 4 children with a present IEM. Such genetic information also offers a basis for genetic counseling in family planning and prenatal diagnostics. Furthermore, it aids the family in preparing for disease onset and progression and it can prevent numerous unnecessary diagnostic procedures [34]. There are still some drawbacks when using NGS testing in NBS, mainly the time-consuming procedure and the often complex interpretation of results [9]. In diseases caused by variants in multiple genes, especially when the genes have many exons, NGS sequencing proved to be less time-consuming than Sanger sequencing of each individual exon separately. Nevertheless, the established follow-up tests (organic acids in urine, amino acids in plasma, acylcarnitines, amino acids in dried blood spots, and Sanger sequencing of smaller genes) remain quicker than using NGS for the time being. Additionally, an informed consent is currently required for genetic screening [35]. In our study, we used whole blood samples to achieve better coverage with the NGS sample preparation kits. A simpler procedure would start the NGS testing from DBSs. Such methods have significant advantages and are now being used more frequently [36]. Genetic testing with a DNA sample from the same DBS as used in first-tier NBS would significantly reduce false positive results and it would also prevent unnecessary stress for families due to recalls. However, in our current setting NGS will only be used for confirmation of metabolic results.

The incidence of a specific IEM is very important when deciding on the inclusion of the disease in an NBS program. The estimation of the cumulative incidences in Slovenia is based on the pilot study and the clinically detected cases. This approach provides the best estimation of the incidences, but it has some limitations. The estimation of the incidences based on clinically detected cases is probably too low, because some cases are missed due to the deaths of undiagnosed patients and some patients could remain undiagnosed [37]. The estimation from the pilot study will also be more accurate as more newborns will be screened. For the Slovenian population, the estimated cumulative incidence of IEMs that are to be included in the expanded NBS program is 1:2762. This is comparable to the Austrian NBS program with a declared incidence rate of 1:2855 [38] and higher than the German incidence of 1:4100 [39]. There is sparse information on the incidences in other countries in Central Europe, although they have established expanded NBS programs [6,40]. Incidences for specific IEMs vary, especially for 3-MCC, VLCAD and MCAD. 3-MCC has a very high estimated incidence, partially supported by the relatively high frequency of heterozygous carriers that have been discovered. The incidence of VLCAD is a rough estimation and the real incidence is probably lower as we did

not find other VLCAD cases in the population of clinically confirmed IEM cases, therefore our estimation is probably too high.

5. Conclusion

The expansion of the current NBS programs is one of the major goals of health-care programs in south-eastern Europe. Our study demonstrates that cumulative incidences of IEMs in Slovenia were similar to the incidences in countries where expanded NBS has been routinely running for years. As a form of confirmatory testing, NGS proved to be a valuable tool in explaining abnormal metabolite concentrations in DBSs and differentiating between affected patients and those with merely a heterozygotic genetic variant. The implementation of NGS as a first follow-up test after the discovery of elevated metabolites in DBSs would improve the NBS programs, reduce the burden of false positive results and lower the number of false negative results at recall.

Conflict of interest

Authors declare no conflict of interest.

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SPREMNA BESEDA

Spoštovane kolegice pediatrinje in kolegi pediatri!

Najprej bi se Vam želeli najlepše zahvaliti za Vaše dosedanje zavzeto delo pri zgodnjem odkrivanju otrok s povišanimi vrednostmi holesterola, zlasti tistih z družinsko obliko hiperholesterolemije. Letno v sklopu tega programa odkrijemo nekaj deset otrok, ki jim je nato genetsko potrjena družinska hiperholesterolemija, ter pri vsakem otroku še enega od staršev in pogosto druge svojce.

Ob rob navodilu za izvajanje tega programa bi želeli jasno poudariti velik pomen teh naših skupnih naporov. Osebe z družinsko hiperholesterolemijo, katerih odkritje je glavni cilj programa presejanja, imajo namreč izrazito, lahko tudi do 100-krat večje tveganje za zgodnji razvoj bolezni srca in ožilja, ki pogosto brez predhodnih opozoril prizadenejo ljudi v najbolj aktivnih letih življenja. Družinsko hiperholesterolemijo ima po novejših ocenah skoraj vsak dvestoti posameznik; s tem gre za daleč najpogostejšo prirojeno bolezen presnove, ki pa je tudi v razvitem svetu izrazito poddiagnosticirana. Skladno s temi ocenami ima v Sloveniji družinsko hiperholesterolemijo med 1000—2000 otrok in mladostnikov. Kljub priporočilom najpomembnejših mednarodnih strokovnih forumov za presejanje v otroštvu, v Sloveniji trenutno še vedno kot edini v svetovnem merilu uspešno populacijsko presejamo hiperholesterolemijo, zaradi česar v zadnjem času prejemamo tudi veliko pozitivne mednarodne pozornosti. V letu 2017 smo vzpostavili Nacionalni register oseb z družinsko hiperholesterolemijo in redkimi dislipidemijami.

Med glavnimi razlogi za presejanje družinske hiperholesterolemije že v otroštvu so: odkrivanje bolezni še preden se izrazijo klinični znaki, ki jih lahko učinkovito preprečujemo s preventivnimi ukrepi, med katere sodita predvsem vzgoja za zdrav življenjski slog in pravočasna uvedba terapije; na podlagi vrednosti holesterola lahko najučinkoviteje razlikujemo družinsko od večfaktorske oblike hiperholesterolemije pri predpubertetnih otrocih; učinkovit sistem sistematskih pregledov v otroštvu, ki zajame celotno populacijo otrok v enakih starostnih obdobjih; s kaskadnim presejanjem pri vsakem otroku s potrjeno družinsko hiperholesterolemijo lahko odkrijemo bolezen tudi pri starših, sorojencih in/ali drugih sorodnikih.

V želji, da bi nadaljevali s kakovostnim in poenotenim delom na tem področju, smo Vam v pomoč pripravili praktično navodilo (algoritem) presejanja hiperholesterolemije v sklopu sistematskega pregleda 5-letnikov oz. otrok pred vstopom v šolo in nadaljnje obravnave otrok z ugotovljeno hiperholesterolemijo. Ker je glede priporočene diete pogosto potreben nasvet, smo pripravili priročna dietna navodila s primeri konkretnih jedilnikov, ki jih boste lahko delili družinam Vaših pacientov.

Opozorili bi radi tudi na praviloma povsem spregledano populacijo oseb s prenizkimi vrednostmi holesterola, med katerimi se med posamezniki z ugodnim kardiovaskularnim profilom tveganja skrivajo posamezni bolniki z resnimi prirojenimi presnovnimi in sindromskimi stanji — ta del (v luči dejstva, da Vam bo izvid testiranja na voljo) dodajamo v algoritem presejanja.

V želji po nadaljnjem dobrem strokovnem sodelovanju Vas lepo pozdravljamo. V primeru praktičnih dilem v zvezi z izvajanjem tega programa presejanja pa smo Vam z veseljem na voljo za posvet.

Algoritem za izvajanje programa presejanja za holesterol pri predšolskih otrocih in prehranske smernice / izdal: Klinični oddelek za endokrinologijo, diabetes in presnovne bolezni, Pediatrična klinika, UKC Ljubljana / urednik: doc. dr. Urh Grošelj, dr. med. / oblikovala: Špela Goltes / lektorirala: Katarina Grabnar / tisk: Garamond / naklada: 1500 izvodov / 1. izdaja / julij, 2017

O ZGIBANKI IN PROGRAMU PRESEJANJA

Program presejanja hiperholesterolemije poteka od leta 1995 kot obvezni del sistematskega pregleda 5-letnikov oz. pred vstopom v šolo. Od leta 2011 na Pediatrični kliniki UKC Ljubljana rutinsko izvajamo genetsko diagnostiko za presejanje družinske hiperholesterolemije.

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- doc. dr. Urh Grošelj, dr. med. (koordinator programa presejanja),
- prof. dr. Tadej Battelino, dr. med.,
- dr. Jernej Kovač, univ. dipl. biokem., in
- mag. Andreja Širca Čampa, univ. dipl. inž., klinični dietetik.

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Razvoj programa presejanja poteka tudi v okviru programa in projektov ARRS (P3-0343, J3-4116 in J3-6798).

Dodatne informacije ali konzultacije v zvezi s programom presejanja:
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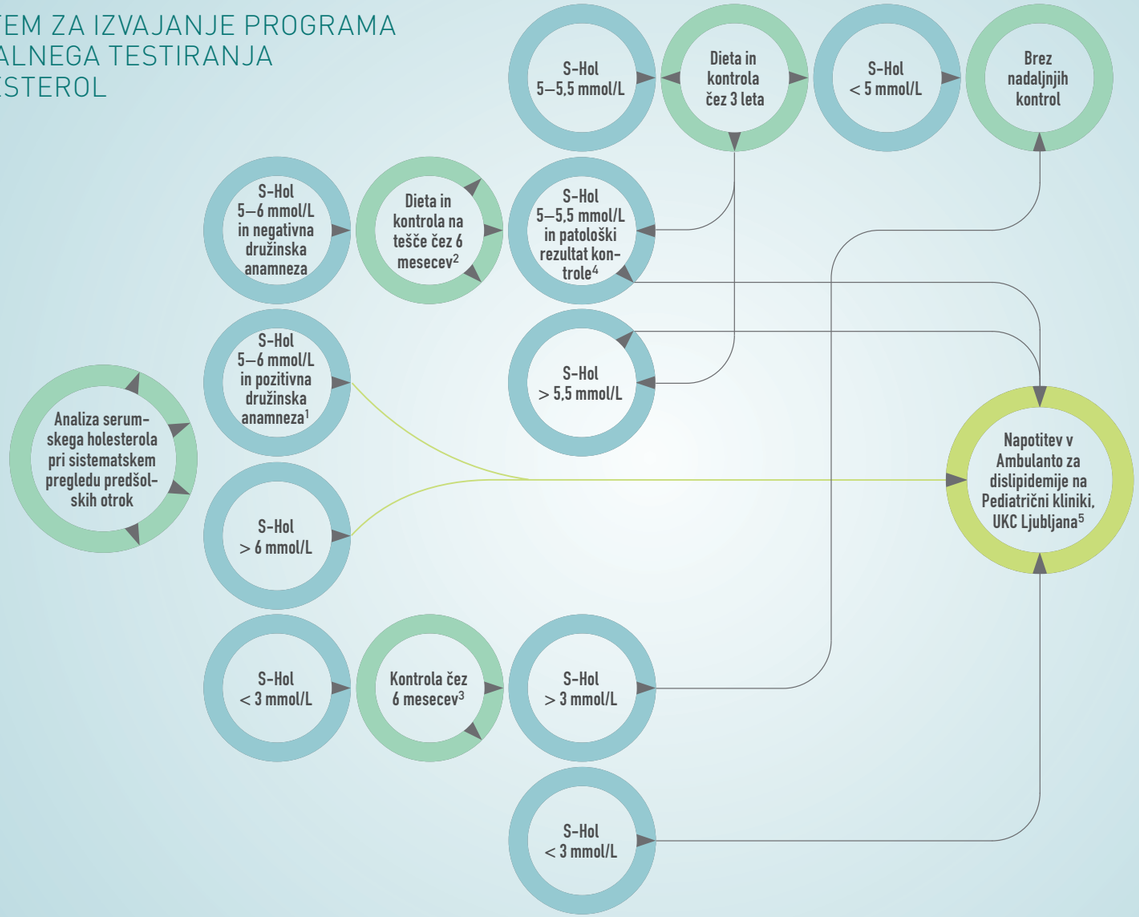
NACIONALNI PROGRAM PRESEJALNEGA TESTIRANJA ZA HOLESTEROL PRI OTROCIH

Algoritem za izvajanje programa presejanja za holesterol pri predšolskih otrocih in prehranske smernice

ALGORITEM ZA IZVAJANJE PROGRAMA PRESEJALNEGA TESTIRANJA ZA HOLESTEROL



1 Pozitivna družinska anamneza: primer izrazitejše hiperholesterolemije pri sorojencih, starših ali starih starših ali primer bolezni srca in ožilja pri starših ali starih starših pred 60. letom starosti. V primeru potrjene družinske hiperholesterolemije pri sorojencih, starših ali starih starših svetujemo napotitev v našo ambulanto.

2 Kontrolni odvzem: S-Hol, S-LDL, S-HDL, S-triglicеridi, S-AST, S-ALT.

3 Ko otrok nima akutne okužbe. Kontrolni odvzem: S-Hol.

4 Dodaten patološki laboratorijski izvid (S-HDL, S-triglicеridi, S-AST, S-ALT) ali prisoten drug izrazit dejavnik tveganja (npr. debelost, arterijska hipertenzija, (pred) diabetes).

5 Ob napotitvi prosimo priložite kopije dosedanjih laboratorijskih meritev holesterola ali pripišite rezultate in datume meritev.

PREHRANA PRI POVIŠANEM HOLESTEROLU

Splošna navodila

Prehrana pri povišanem holesterolu pri idealni ali normalni telesni teži temelji na energijsko in hranilno uravnoteženi prehrani in na pravilnem režimu prehranjevanja:

- Energijski vnos je v ravnovesju s porabljeno energijo.
- Od 4 do 5 rednih obrokov dnevno.
- Celokupni holesterol v prehrani pod 200 mg/dan.
- Delež vseh maščob 25—30 % dnevnega energijskega vnosa.
- Delež nasičenih maščob do 7 % dnevnega energijskega vnosa.
- Delež beljakovin 15 % dnevnega energijskega vnosa.
- Delež ogljikovih hidratov 55 % dnevnega energijskega vnosa.
- Prehranske vlaknine 17—30 g/dan, od tega 7—13 g topnih.
- Rastlinski steroli 2—3 g/dan.
- Vključiti polnovredna živila, sadje, zelenjavo in stročnice.
- Od 3 do 4-krat tedensko mastne morske ribe.
- Prehrana z manj maščobami in zmerna uporaba vseh živil, bogatih z maščobo.
- Omejiti vnos nasičenih maščob in pri izbiri dati prednost enkrat in večkrat nenasičenim maščobam.
- Omejiti uporabo rafiniranih in s sladkorjem bogatih živil.

Priporočena živila

Živila, bogata s topno prehransko vlaknino: soja, fižol, oves, suhe marelice in slive, prosena kaša, rž, čebula, semena in oreščki, sadje in zelenjava, polnovredna živila.

Živila, bogata z omega-3-maščobnimi kislinami: mastne ribe (losos, slanik, sardine, sardele, skuša, tuna, papalina, palamida), repično olje, laneno olje, orehovo olje, sojino olje.

Živila, bogata z rastlinskimi steroli: rastlinska olja, semena in oreščki, margarine z dodanimi steroli.

Živila, bogata s sojinimi beljakovinami: soja, tofu, sojin napitek.

Odsvetovana živila

Živila, bogata z nasičeno maščobo: kokosova maščoba, palmina maščoba, maslo, goveje salo, svinjska mast, mastno meso, piščančja maščoba, rumenjak, smetana, siri, trda margarina, mlečna čokolada.

Živila, bogata s transmaščobnimi kislinami: mastna govedina, maslo, mlečne maščobe, trde margarine, torte, peciva, piškoti, krekerji, ocvrto pecivo.

Živila, bogata s holesterolom: drobovina, jajčni rumenjak, maslo, smetana, piščančja koža, mehkužci in školjke, jetrna pašteta, krvavice, trda margarina, mastna peciva.